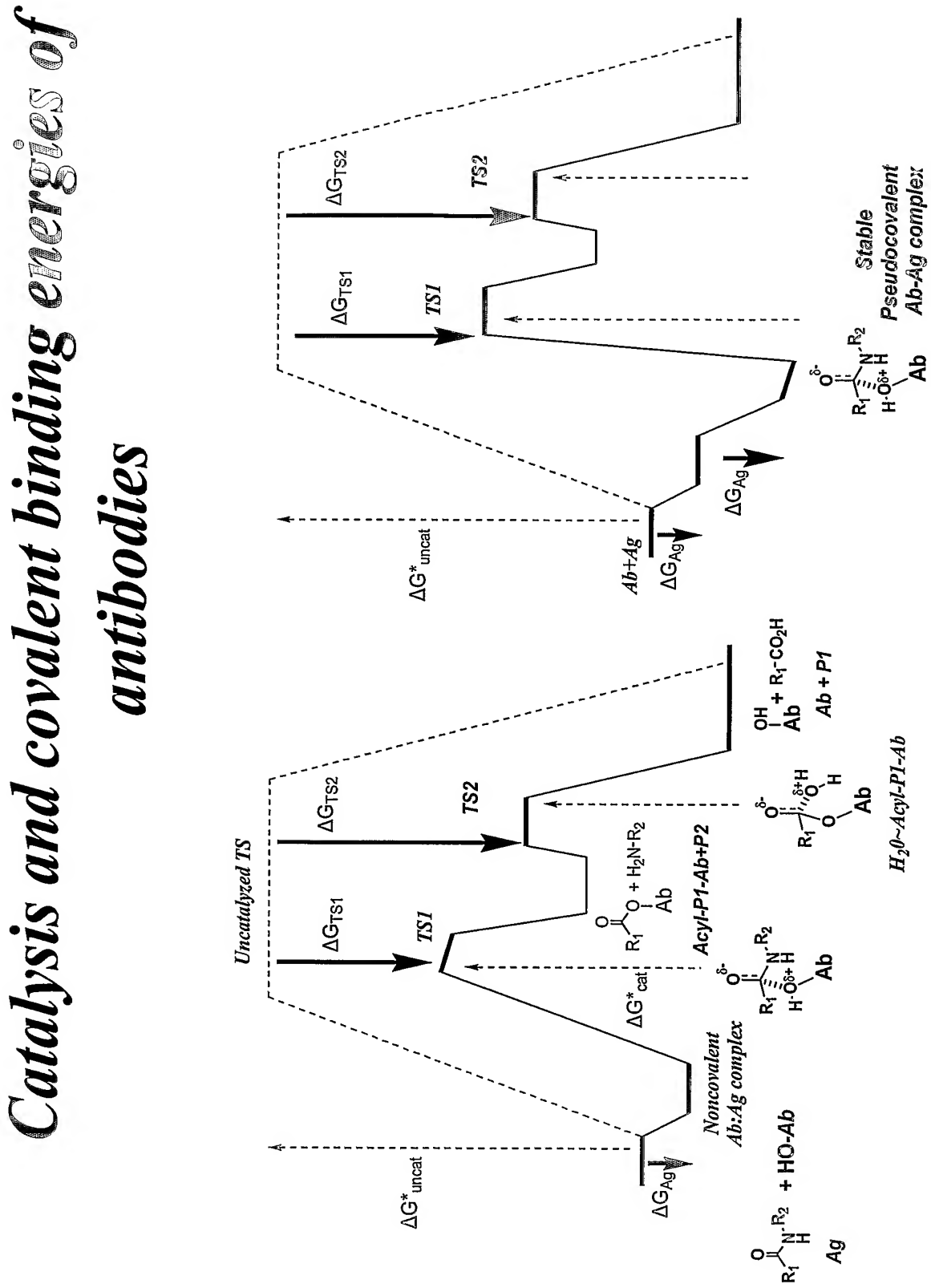

Catalysis and covalent binding energies of antibodies
Uncatalyzed TS
ΔG*uncat
ΔGTS1
TS1
ΔGTS2
TS2
ΔG*cat
ΔGAg
Noncovalent Ab:Ag complex
Acyl-P1-Ab+P2
H20~Acyl-P1-Ab
Ab + P1
Ab+Ag
Stable Pseudocovalent Ab-Ag complex
+ HO-Ab
Ag
Ab

Fig 1

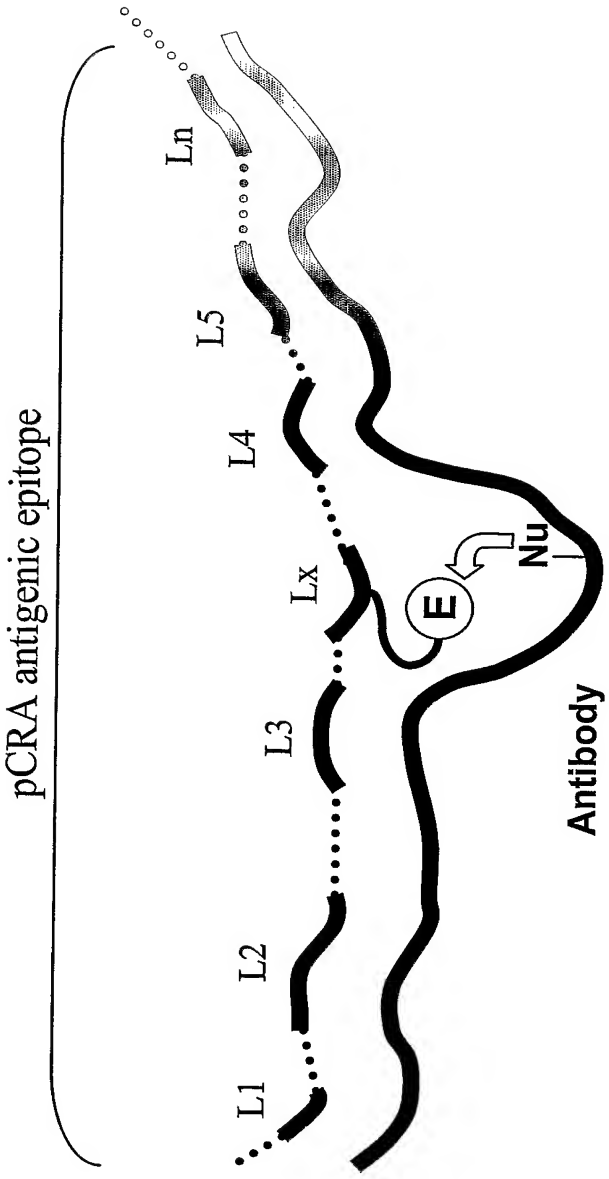
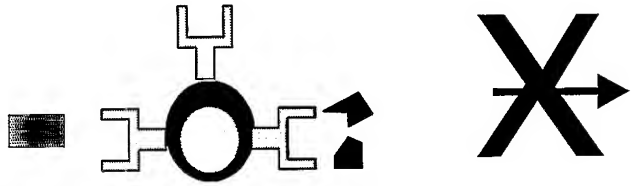


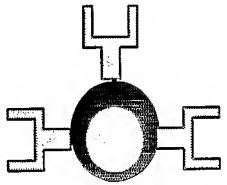
Fig 2

Induction of catalyst synthesis

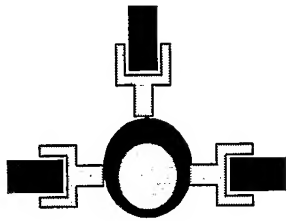
Ordinary polypeptide antigen,
Slow transmembrane signal



Aborted clonal selection,
tolerance



Covalent stimulation,
Rapid transmembrane signal



Increased turnover, proliferation

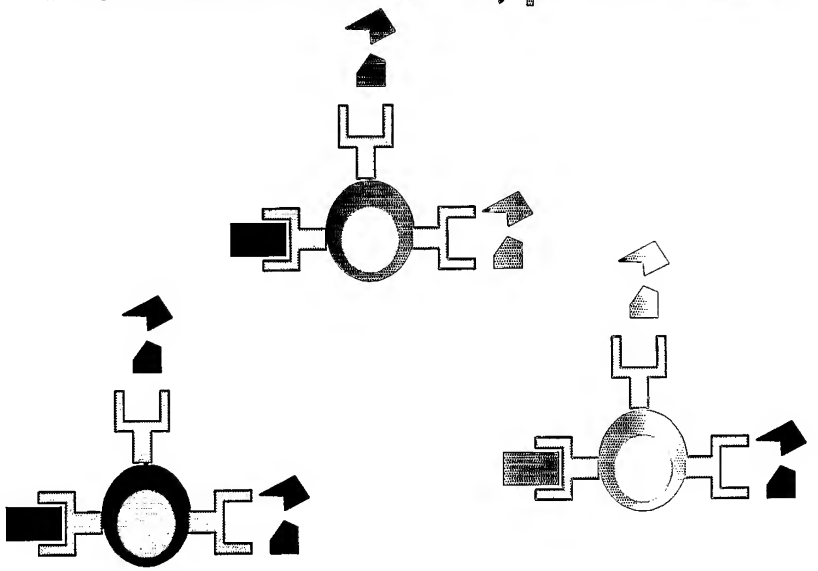
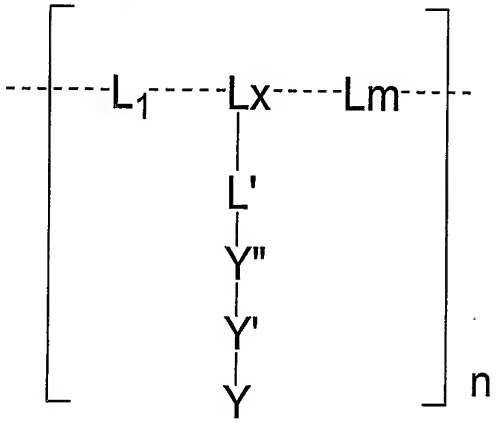


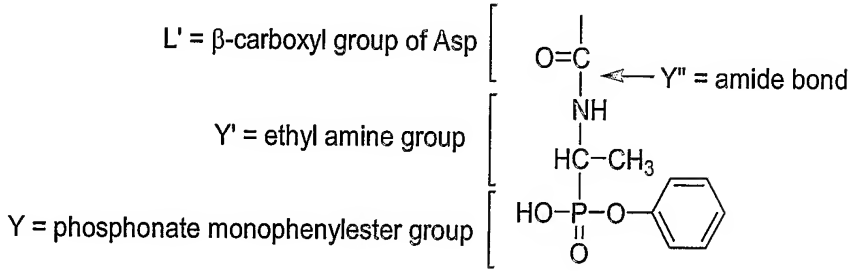
Fig 3

Fig 4

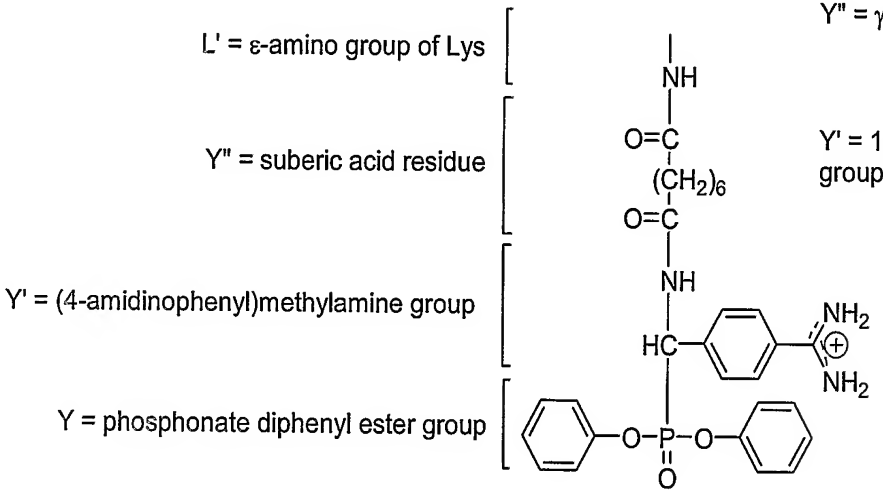
General structure of pCRA



• L'-Y"-Y'-Y, Example 1



• L'-Y"-Y'-Y, Example 2



• L'-Y"-Y'-Y, Example 3

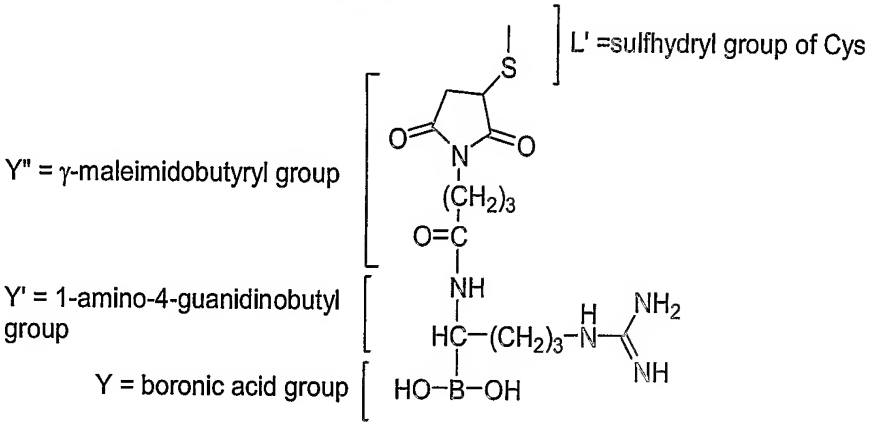
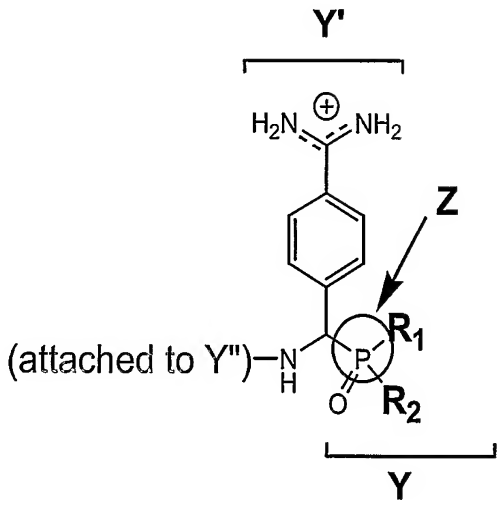
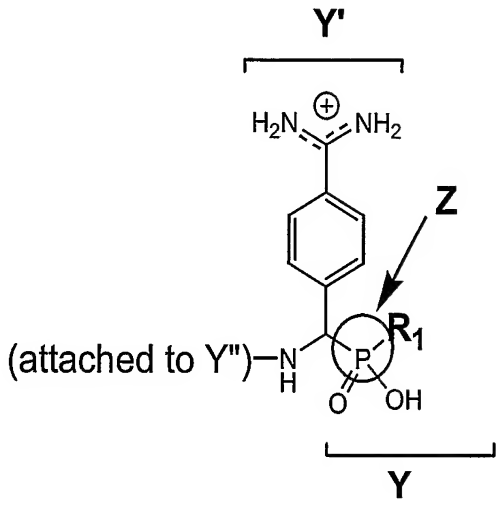


Fig 5A



pCRA Z-R pair: Example 1



pCRA Z-R pair: Example 2

Fig 5B

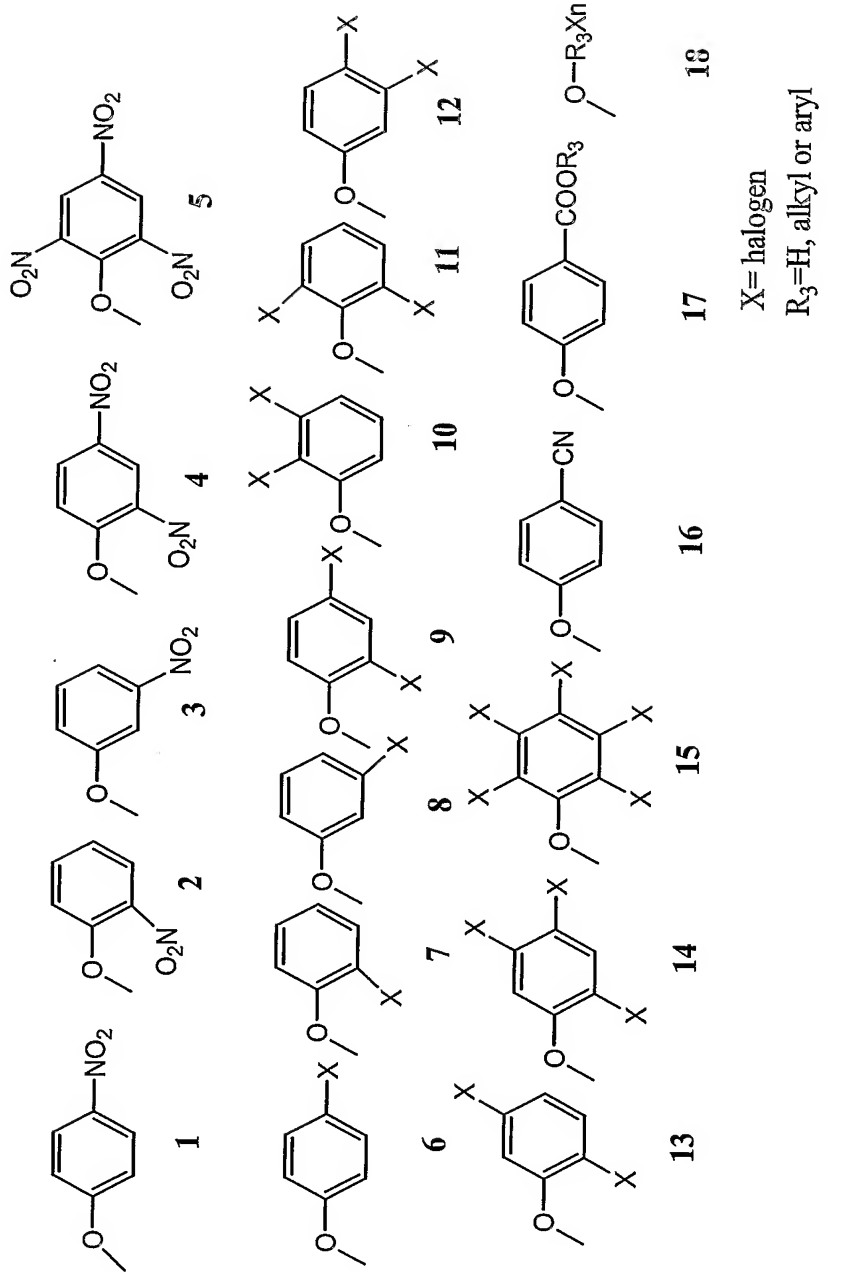


Fig 5C

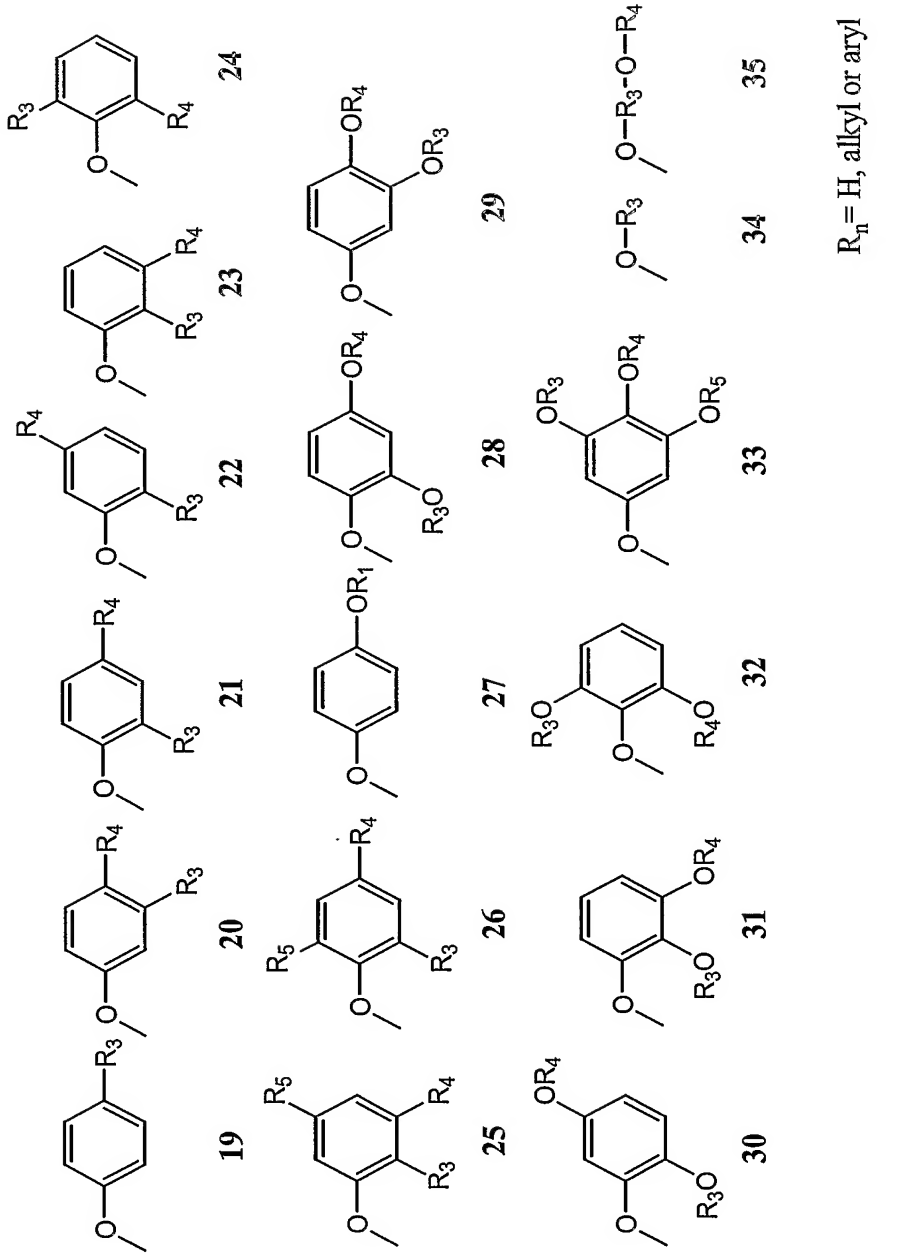
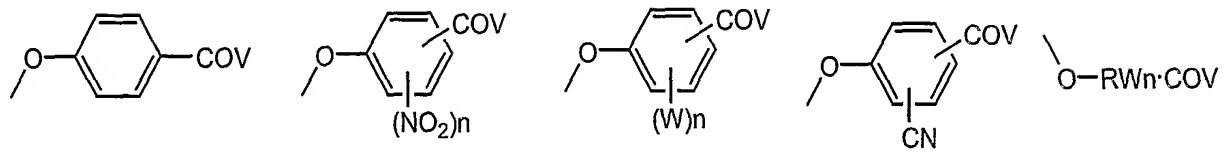


Fig 5D

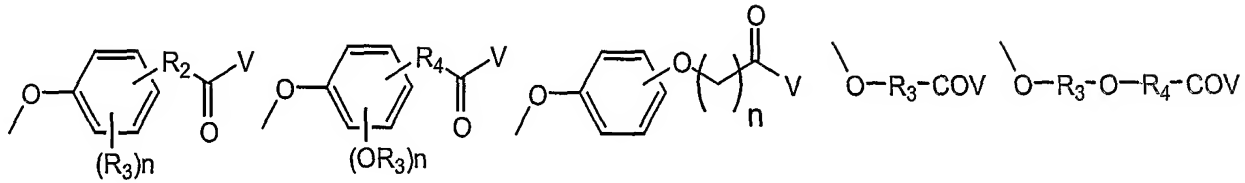
A. Electron withdrawing substituents with peptide extension



36 **37** **38** **39** **40**

W: halogen
V: peptide
R: alkyl or aryl

B. Electron donating substituents with peptide extension



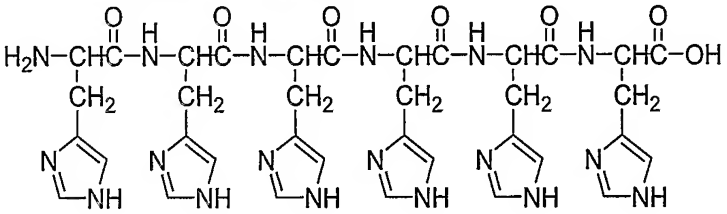
41 **42** **43** **44** **45**

V: peptide
Rn: alkyl or aryl

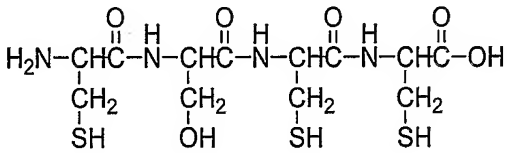
Selected examples of metal binding moiety

1. (His)n
2. (Cys-Aaa-Cys-Cys): metallothionein α-domain-derived peptide
3. (Cys-Aaa-Cys): metallothionein β-domain-derived peptide
4. EDTA
5. a crown ether
6. DAMP

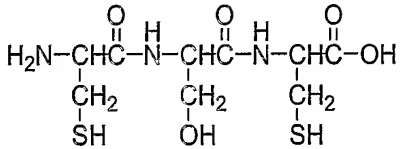
Example of 1: $(His)_6$



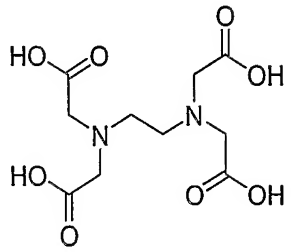
Example of 2: Cys-Ser-Cys-Cys



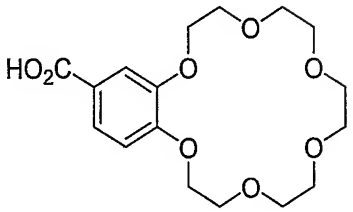
Example of 3: Cys-Ser-Cys



Example of 4: EDTA



Example of 5: 4'-Carboxybenzo-18-crown-6



Example of 6: DAMP

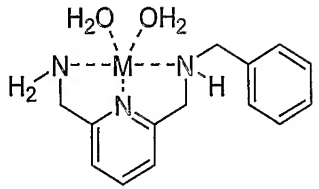


Fig 6

Fig. 7

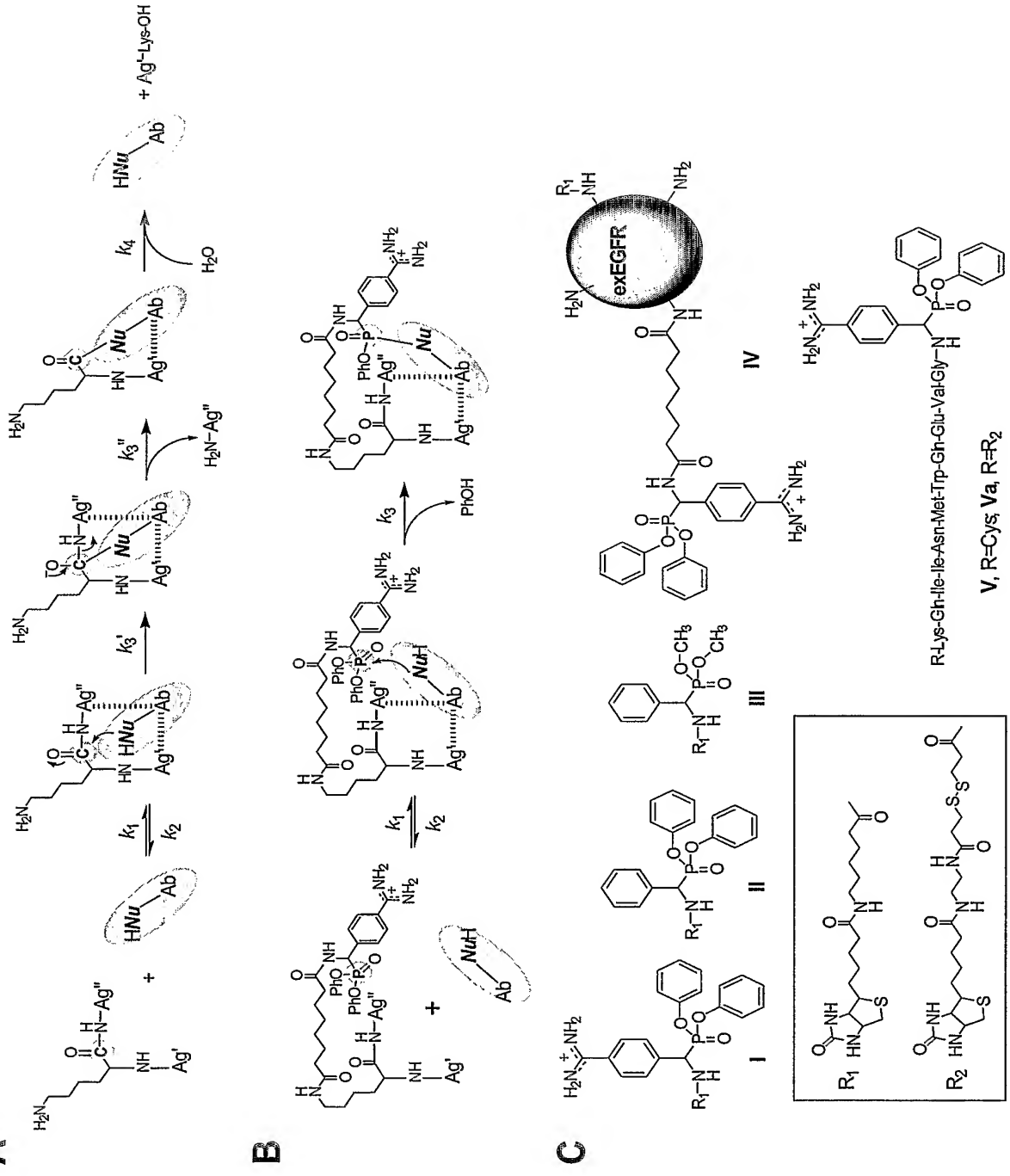

A
B
C
exEGFR
I
II
III
IV
V, R=Cys; Va, R=R2
R-Lys-Gh-Ile-Ile-Asn-Met-Trp-Gh-Glu-Val-Gly-

Fig. 8

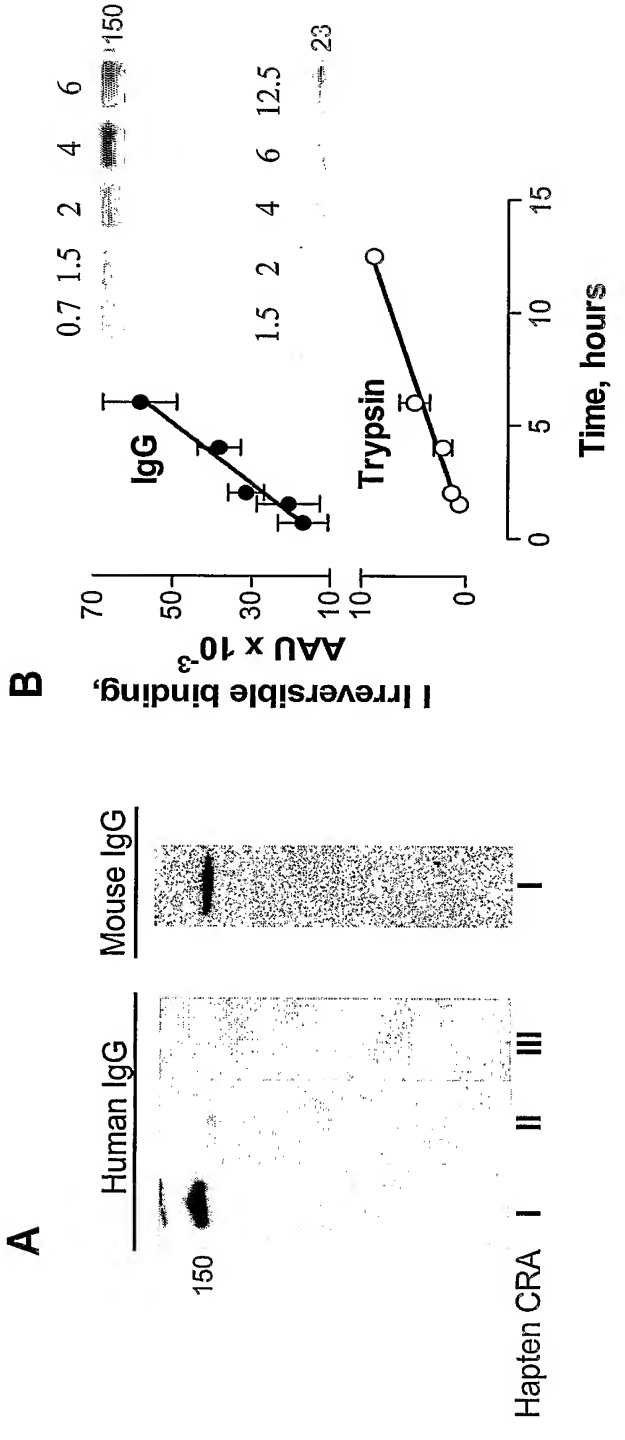

A
Human IgG
Mouse IgG
150
Hapten CRA I II III I
B
Irreversible binding, AAU x 10-3
70
50
30
10
10
0
IgG
Trypsin
0.7 1.5 2 4 6 150
1.5 2 4 6 12.5 23
0 5 10 15
Time, hours

Fig. 9

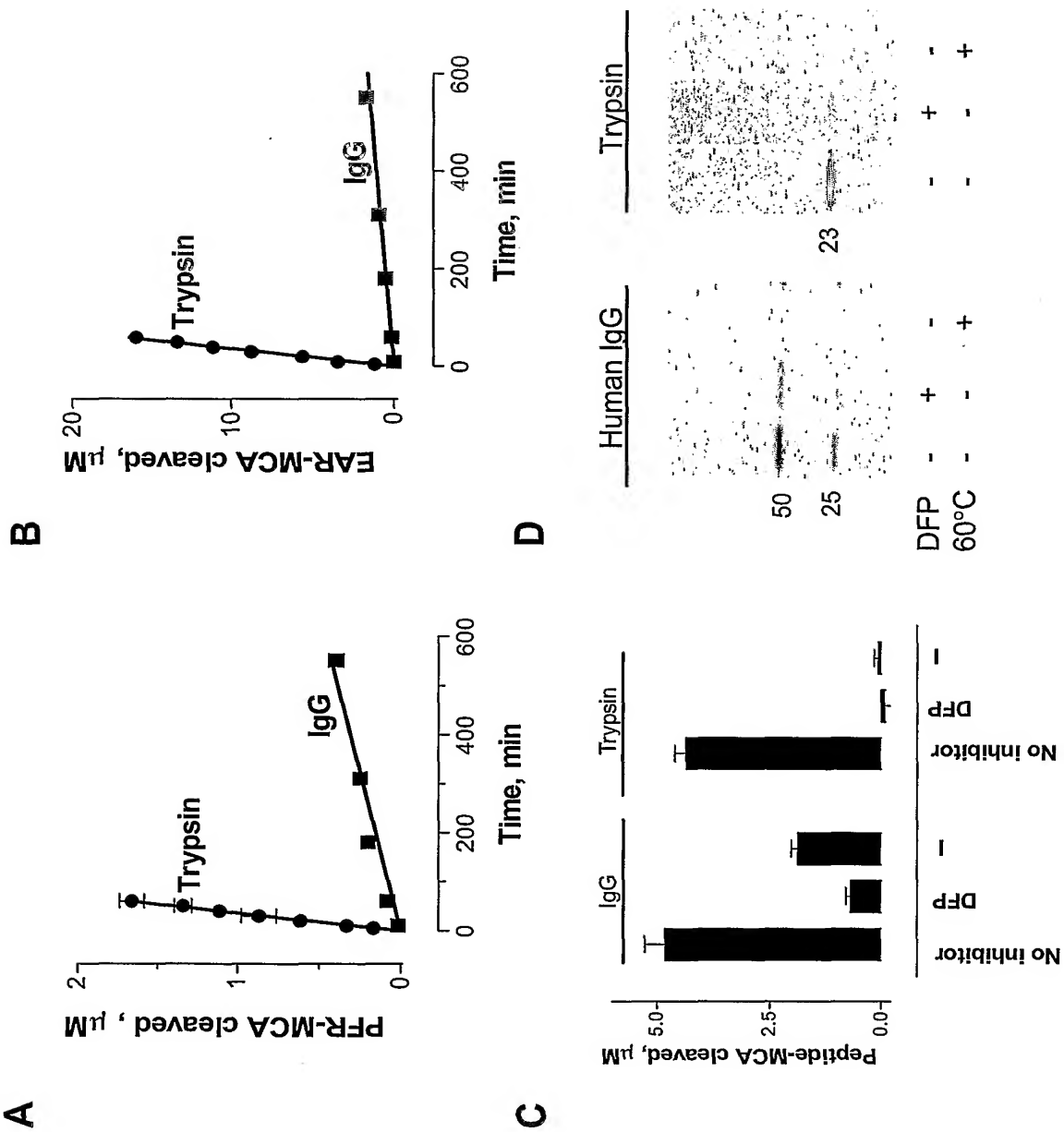
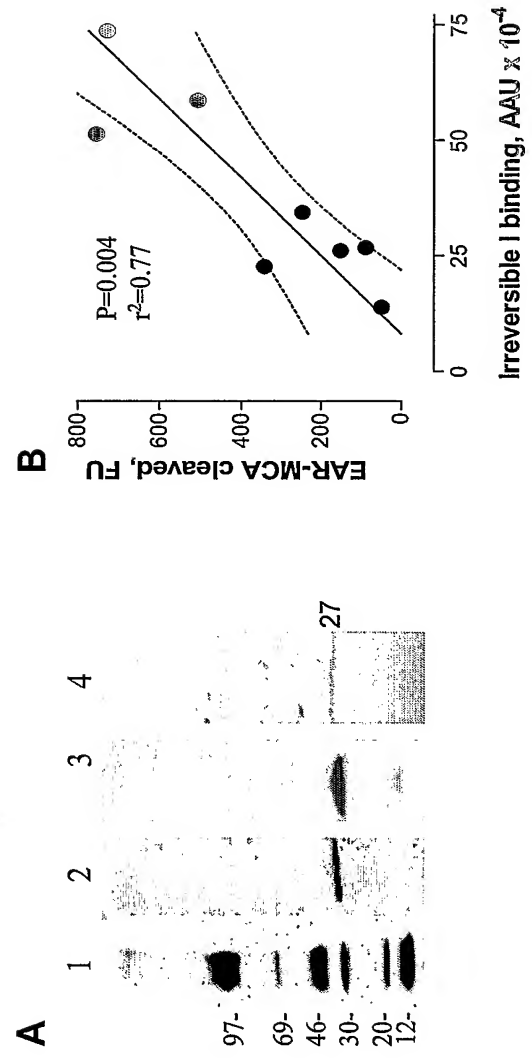

A
PFR-MCA cleaved , μM
Trypsin
IgG
Time, min
B
EAR-MCA cleaved, μM
Trypsin
IgG
Time, min
C
Peptide-MCA cleaved, μM
IgG
Trypsin
No inhibitor
DFP
I
No inhibitor
DFP
I
D
Human IgG
Trypsin
50
25
23
DFP
60°C

Fig. 10


A
1
2
3
4
97-
69-
46-
30-
20-
12-
27
B
EAR-MCA cleaved, FU
800
600
400
200
0
P=0.004
r²=0.77
0
25
50
75
Irreversible I binding, AAU x 10⁻⁴

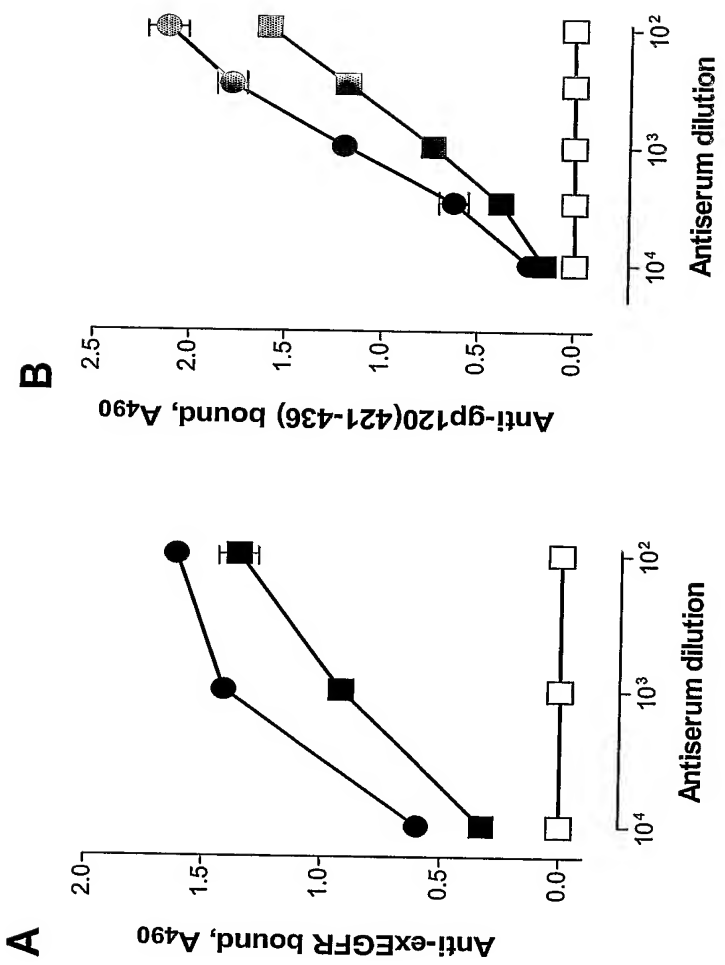

A
Anti-exEGFR bound, A490
2.0
1.5
1.0
0.5
0.0
10^4
10^3
10^2
Antiserum dilution
B
Anti-gp120(421-436) bound, A490
2.5
2.0
1.5
1.0
0.5
0.0
10^4
10^3
10^2
Antiserum dilution

Fig 11

Fig 12

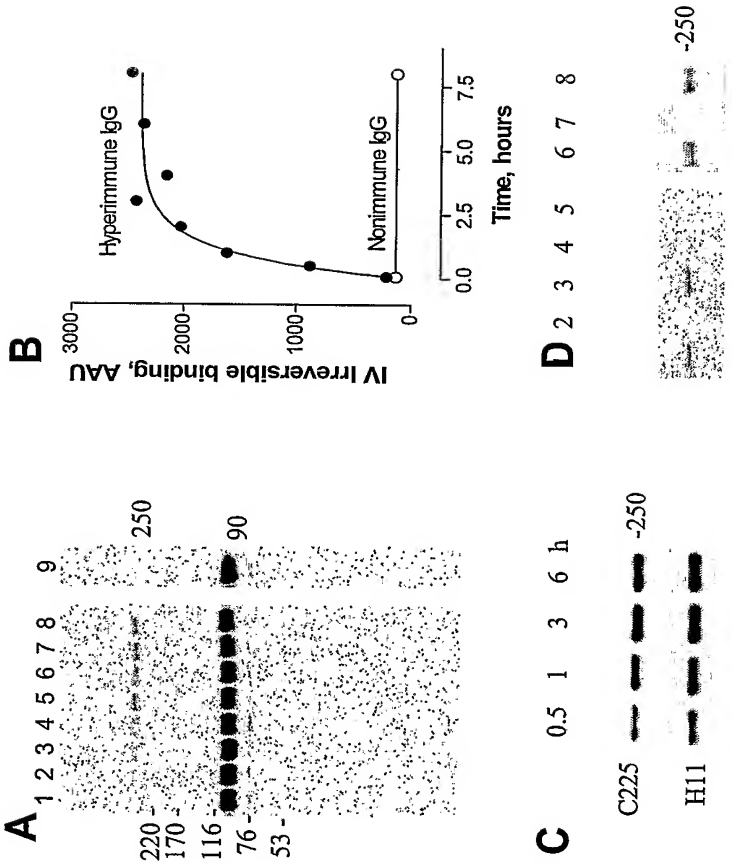

A
1 2 3 4 5 6 7 8 9
220
170
116
76
53
250
90
B
IV Irreversible binding, ΔAU
3000
2000
1000
0
Hyperimmune IgG
Nonimmune IgG
0.0 2.5 5.0 7.5
Time, hours
C
0.5 1 3 6 h
C225
H11
111.6
BGN/H8
D
2 3 4 5 6 7 8
-250
-250

Fig 13

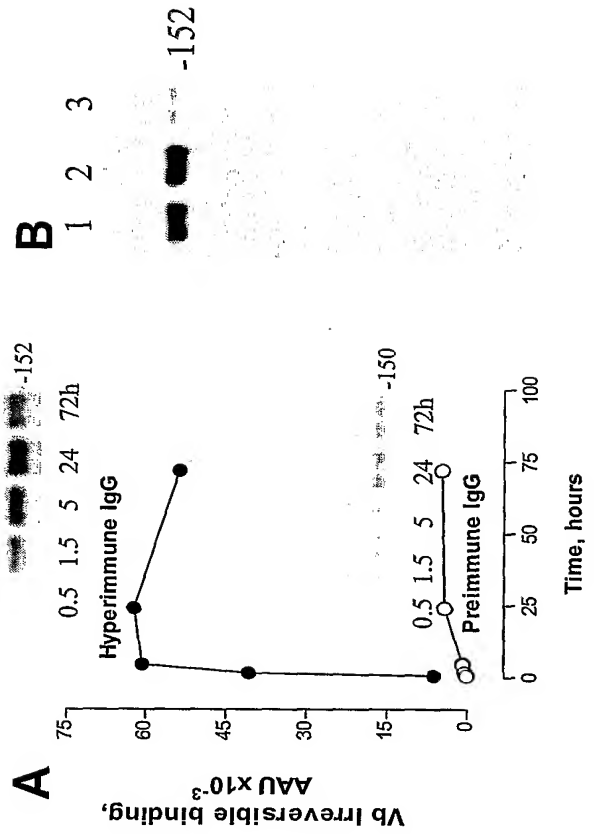

A
B
1 2 3
-152
-150
0.5 1.5 5 24 72h
Hyperimmune IgG
Preimmune IgG
Vb Irreversible binding, ΔAU x10-3
Time, hours
0 25 50 75 100
0 15 30 45 60 75

Fig 14

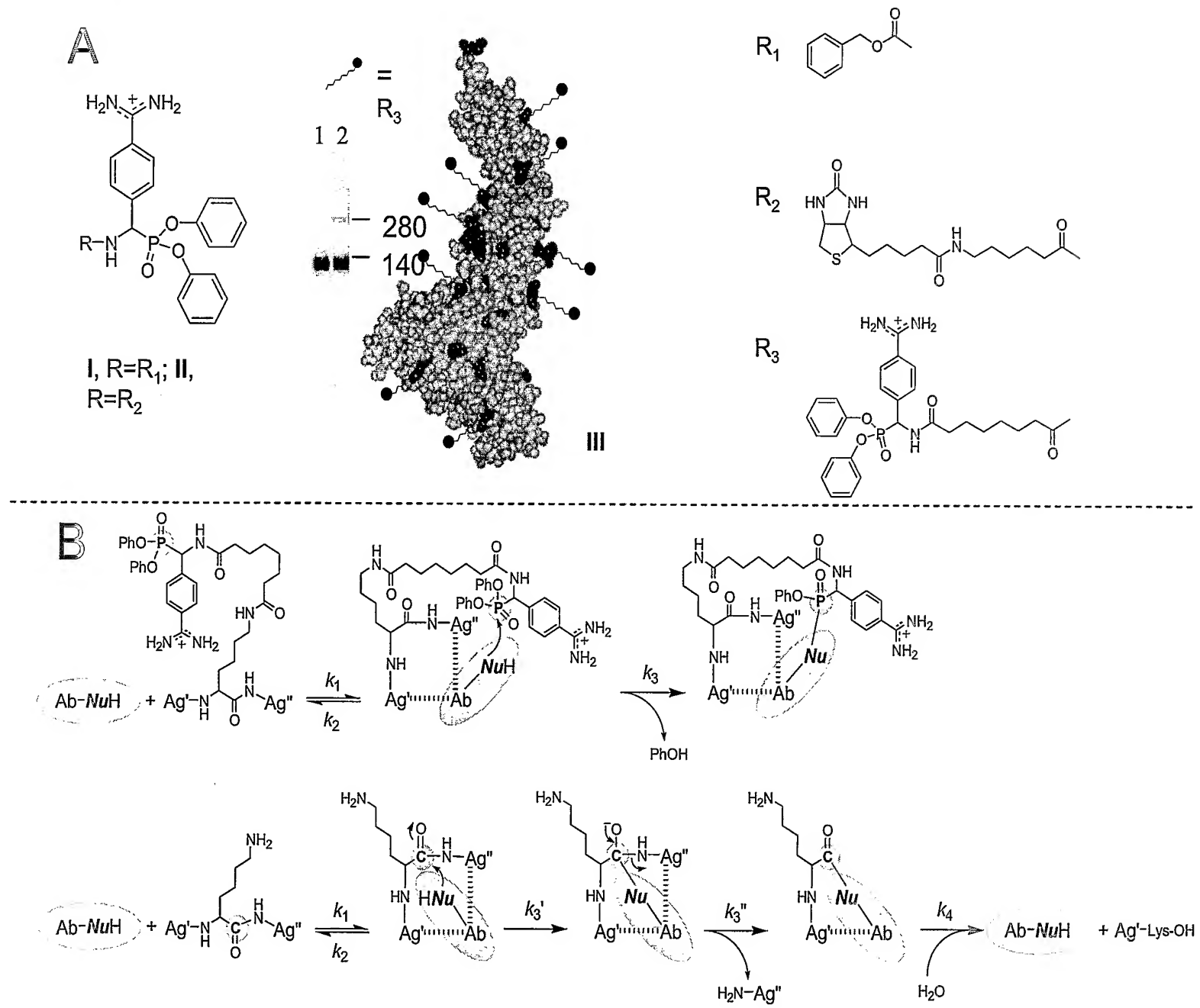

A
I, R=R1; II, R=R2
= R3
1 2
280
140
III
R1
R2
R3
B
Ab-NuH + Ag'-N(H)-C(=O)-N(H)-Ag"
k1
k2
k3
PhOH
k3'
k3"
k4
H2N-Ag"
H2O
+ Ag'-Lys-OH

Fig 15

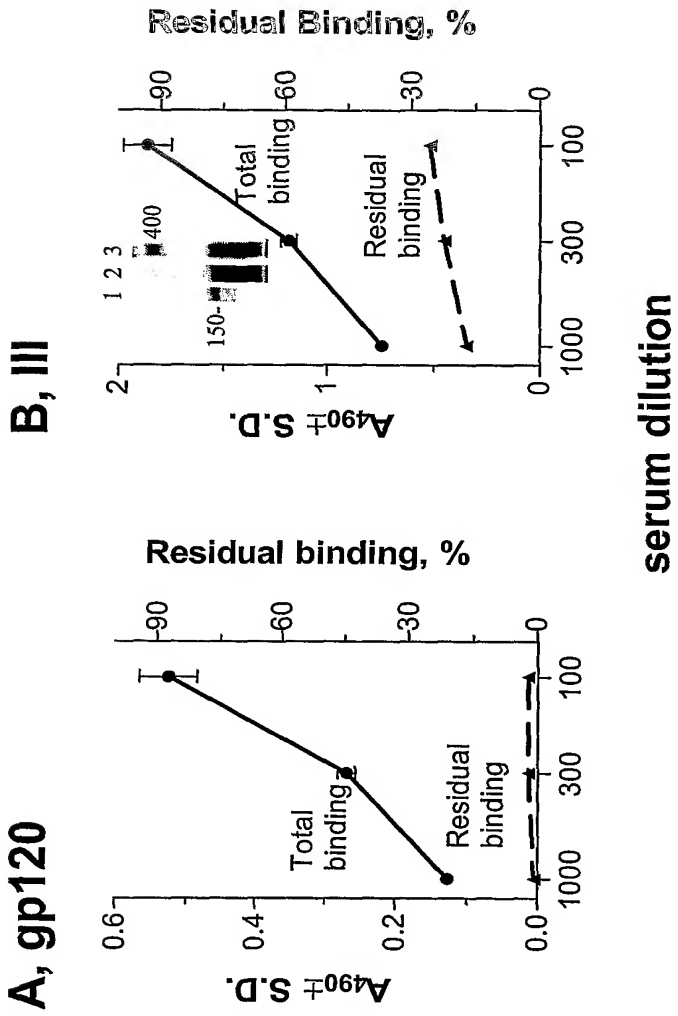

A, gp120
Residual binding, %
A490± S.D.
Total binding
Residual binding
B, III
Residual Binding, %
1 2 3
400
150-
serum dilution

Fig 16

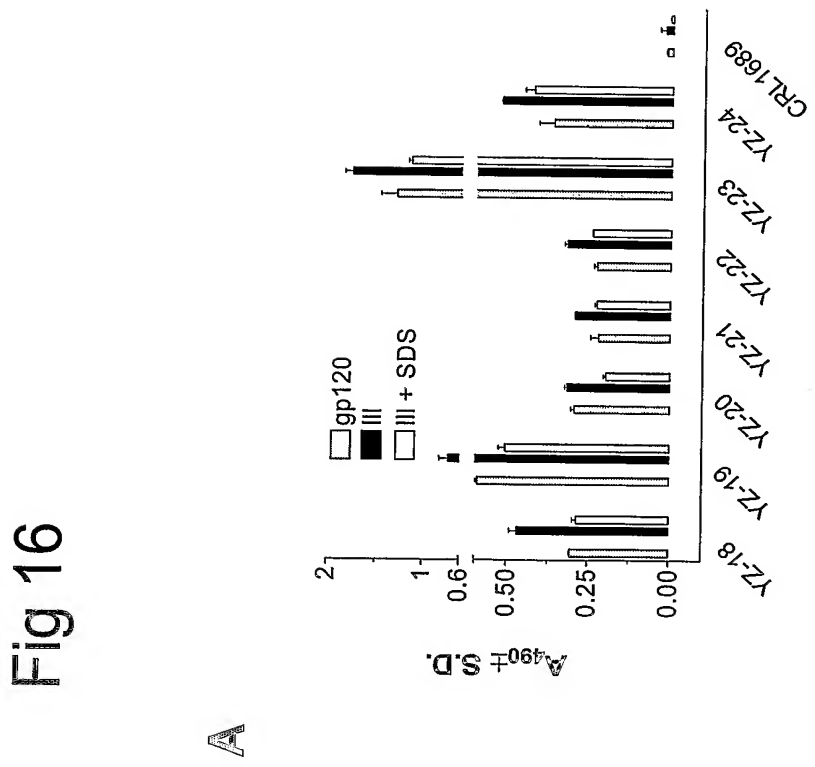

A
A_{490}± s.D.
gp120
III
III + SDS
2
1
0.6
0.50
0.25
0.00
YZ-18
YZ-19
YZ-20
YZ-21
YZ-22
YZ-23
YZ-24
CRL1689
B
A_{490}±S.D.
1.5
1.0
0.5
0.0
gp120
III
III + SDS
0.0
0.5
1.0
[IgG], μM

Fig 17

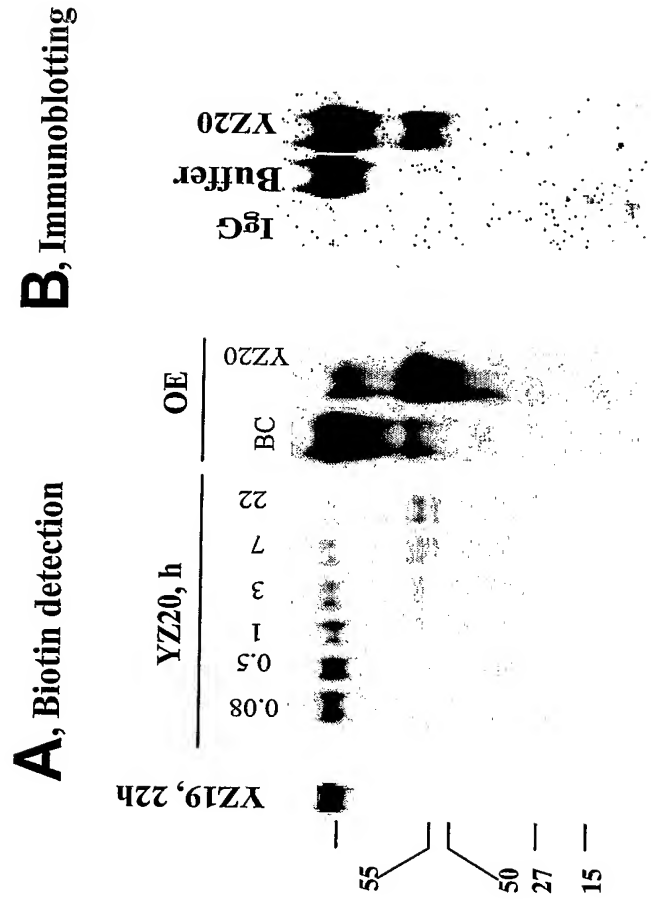

A, Biotin detection
B, Immunoblotting
YZ19, 22h
YZ20, h
0.08
0.5
1
3
7
22
OE
BC
YZ20
IgG
Buffer
YZ20
55
50
27
15

Fig 18

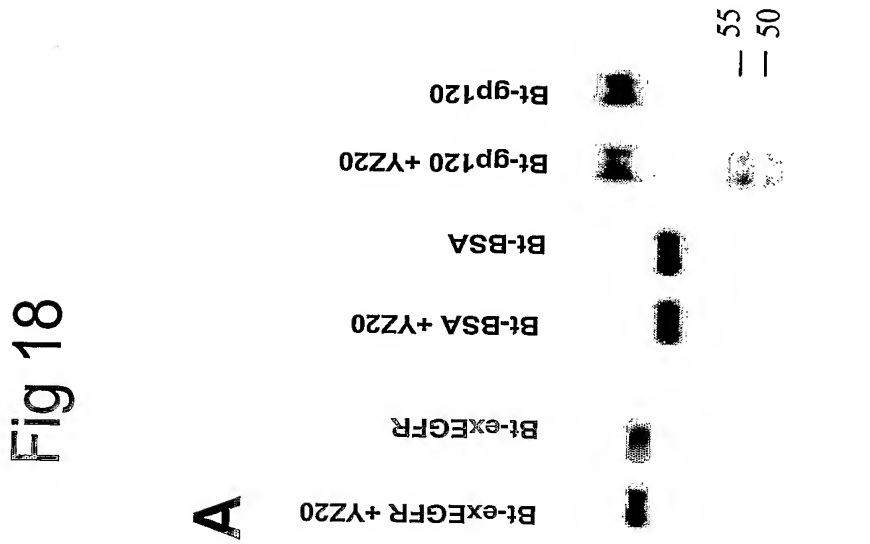

A
Bt-exEGFR +YZ20
Bt-exEGFR
Bt-BSA +YZ20
Bt-BSA
Bt-gp120 +YZ20
Bt-gp120
55
50
B
μM AMC / 22h / μM IgG
0
5
10
15
AE-MCA
AAA-MCA
IIW-MCA
AAPF-MCA
EKK-MCA
VLK-MCA
EAR-MCA
IEGR-MCA
PFR-MCA
C
μM AMC / 19h / μM IgG
0.0
0.1
0.2
0.3
0.4
GGR-MCA
GGL-MCA

Fig 19

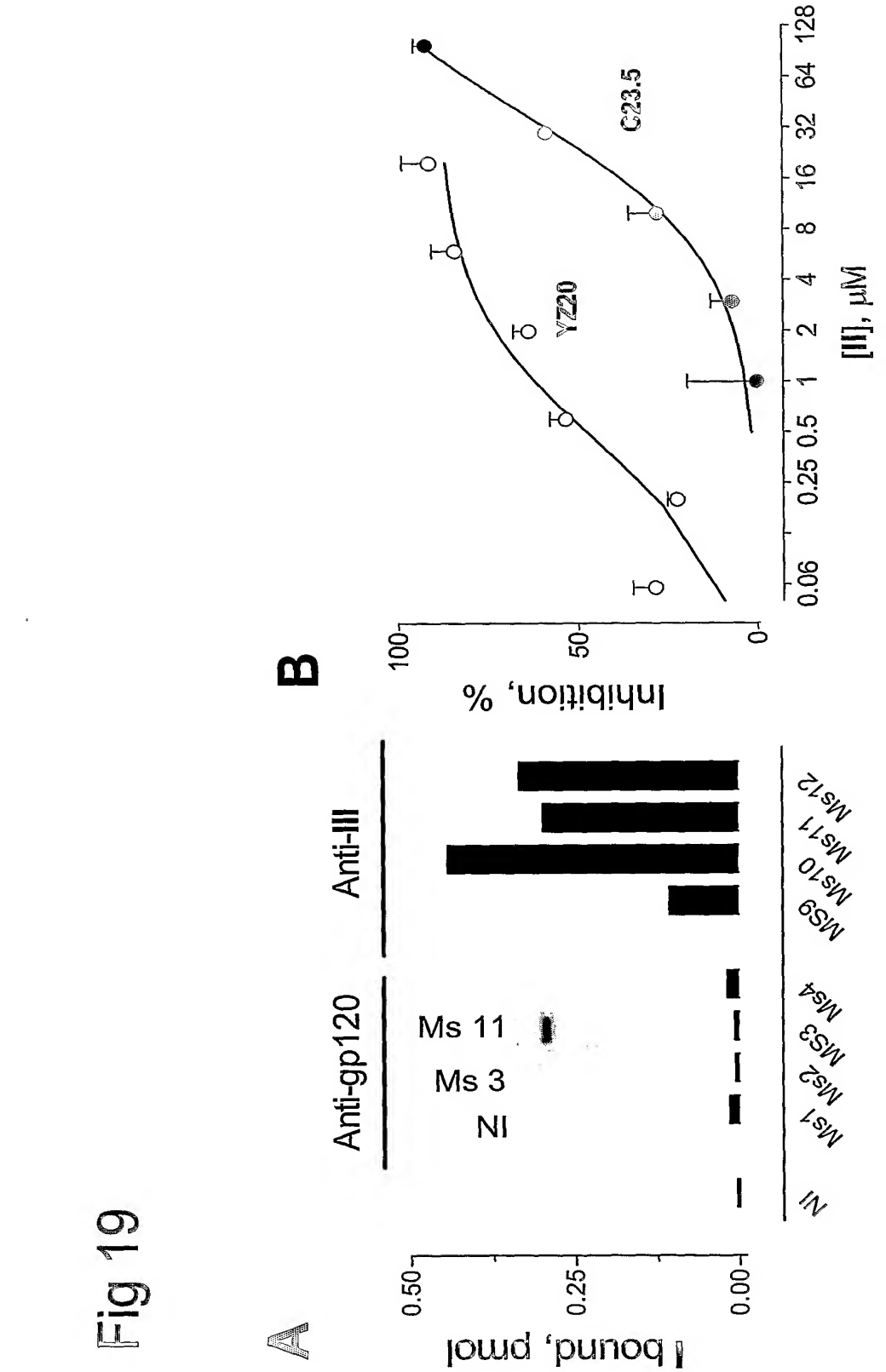
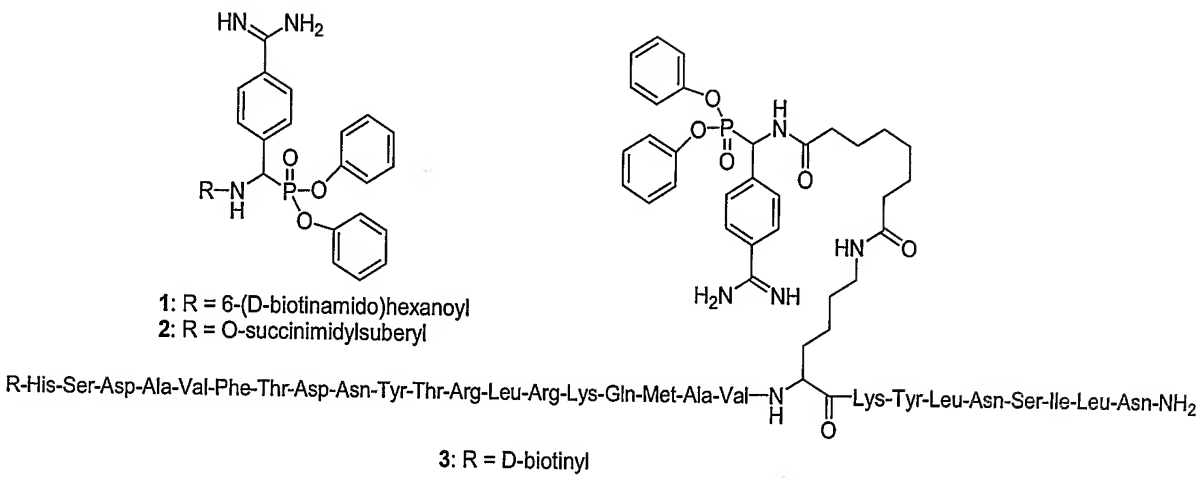

A
B
I bound, pmol
0.50
0.25
0.00
Anti-gp120
Anti-III
Ms 11
Ms 3
NI
NI
Ms1
Ms2
Ms3
Ms4
MS9
Ms10
Ms11
Ms12
Inhibition, %
100
50
0
YZ20
C23.5
0.06
0.25
0.5
1
2
4
8
16
32
64
128
[III], µM

Fig 20

A


HN NH2
R–N H
P
O
O
O
1: R = 6-(D-biotinamido)hexanoyl
2: R = O-succinimidylsuberyl
H2N NH
HN O
R-His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val
Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH2
3: R = D-biotinyl

B

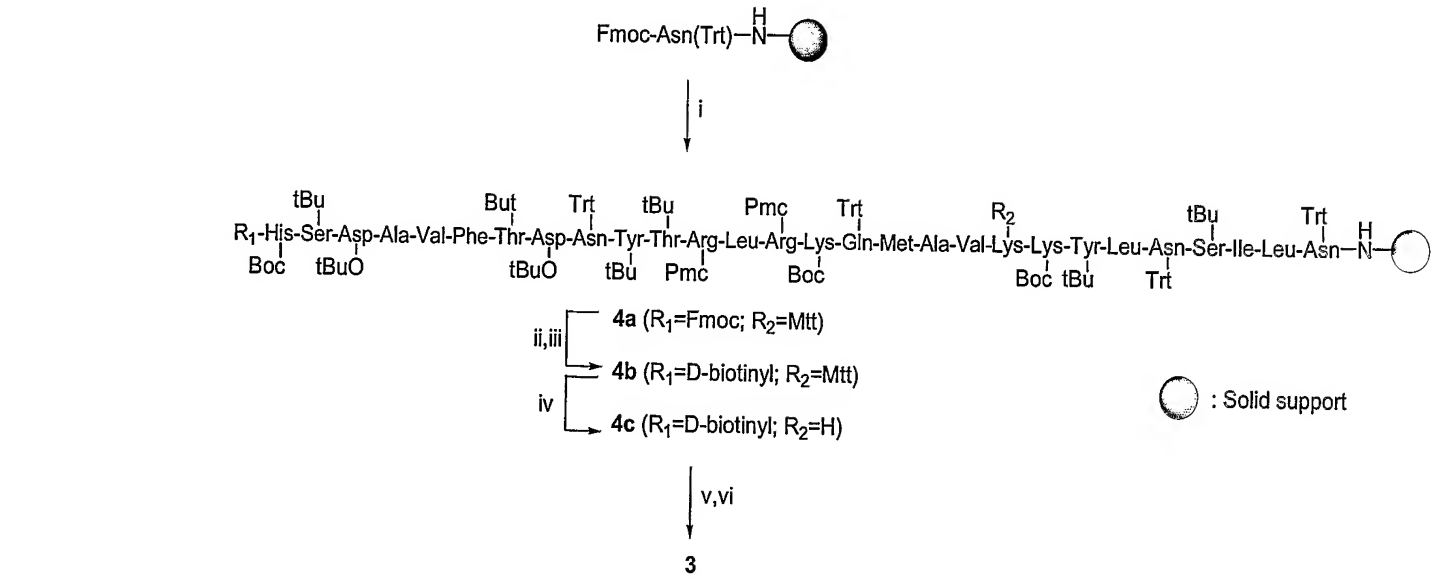

Fmoc-Asn(Trt)
i
R1-His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn
Boc tBuO tBu Boc tBuO tBu Pmc Boc Boc tBu Trt
ii,iii
4a (R1=Fmoc; R2=Mtt)
4b (R1=D-biotinyl; R2=Mtt)
iv
4c (R1=D-biotinyl; R2=H)
: Solid support
v,vi
3

Fig 21

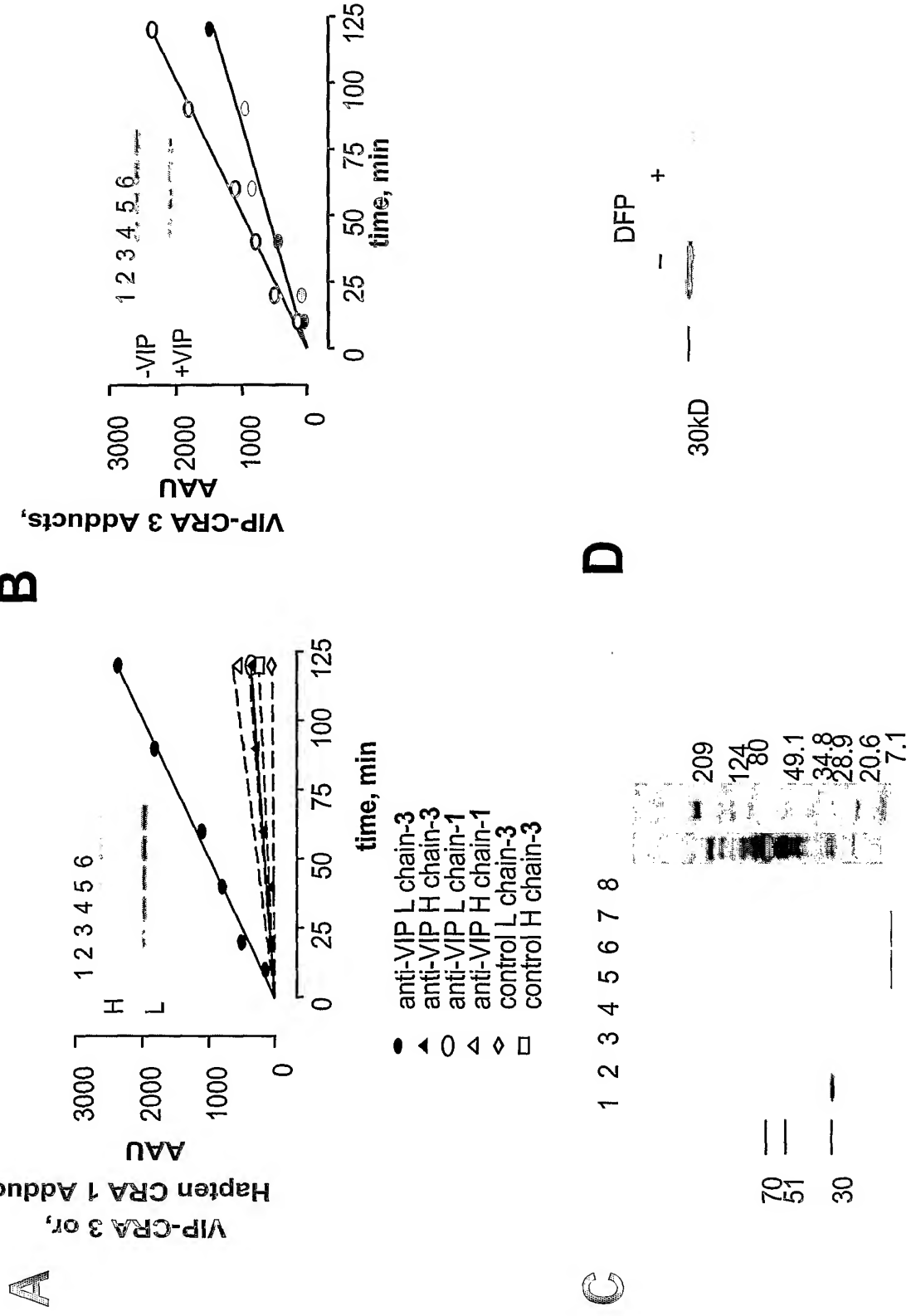

A
VIP-CRA 3 or,
Hapten CRA 1 Adducts
AAU
3000
2000
1000
0
1 2 3 4 5 6
H
L
0 25 50 75 100 125
time, min
anti-VIP L chain-3
anti-VIP H chain-3
anti-VIP L chain-1
anti-VIP H chain-1
control L chain-3
control H chain-3
B
VIP-CRA 3 Adducts,
AAU
3000
2000
1000
0
-VIP
+VIP
1 2 3 4 5 6
0 25 50 75 100 125
time, min
C
1 2 3 4 5 6 7 8
209
124
80
49.1
34.8
28.9
20.6
7.1
70
51
30
D
DFP
–
+
30kD

Fig 22

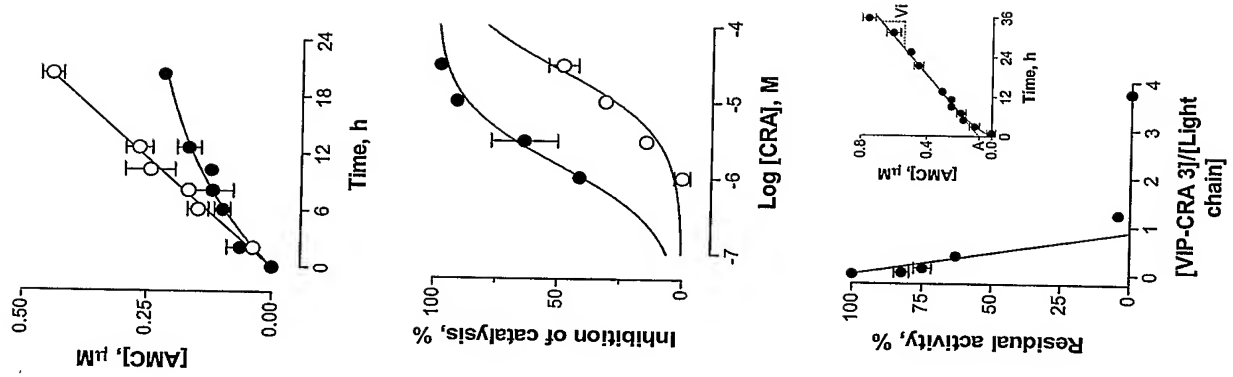
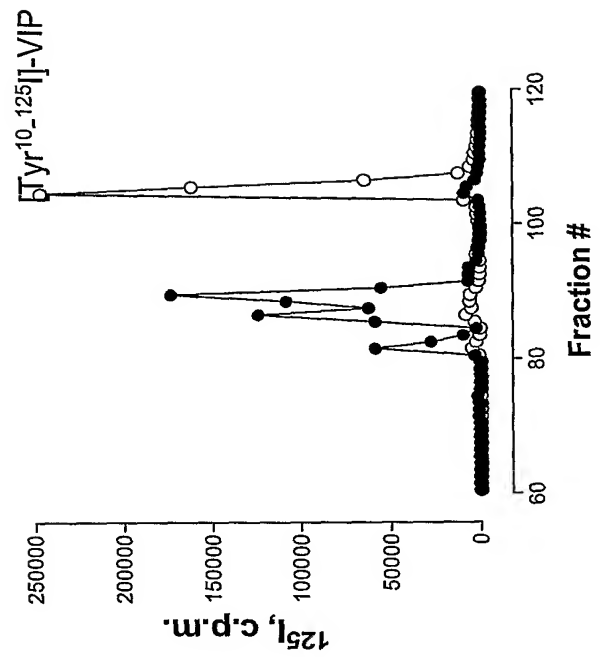

A
[AMC], μM
0.50
0.25
0.00
0
6
12
18
24
Time, h
B
Inhibition of catalysis, %
100
50
0
-7
-6
-5
-4
Log [CRA], M
C
Residual activity, %
100
75
50
25
0
0
1
2
3
4
[VIP-CRA 3]/[Light chain]
[AMC], μM
0.8
0.4
0.0
0
12
24
36
Time, h
Vi

Fig 23


A
[Tyr10_125I]-VIP
125I, c.p.m.
250000
200000
150000
100000
50000
0
60
80
100
120
Fraction #

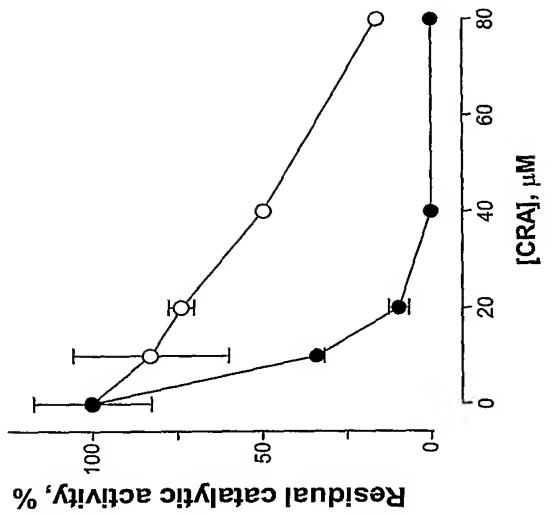

B
Residual catalytic activity, %
100
50
0
0
20
40
60
80
[CRA], μM

Fig 24

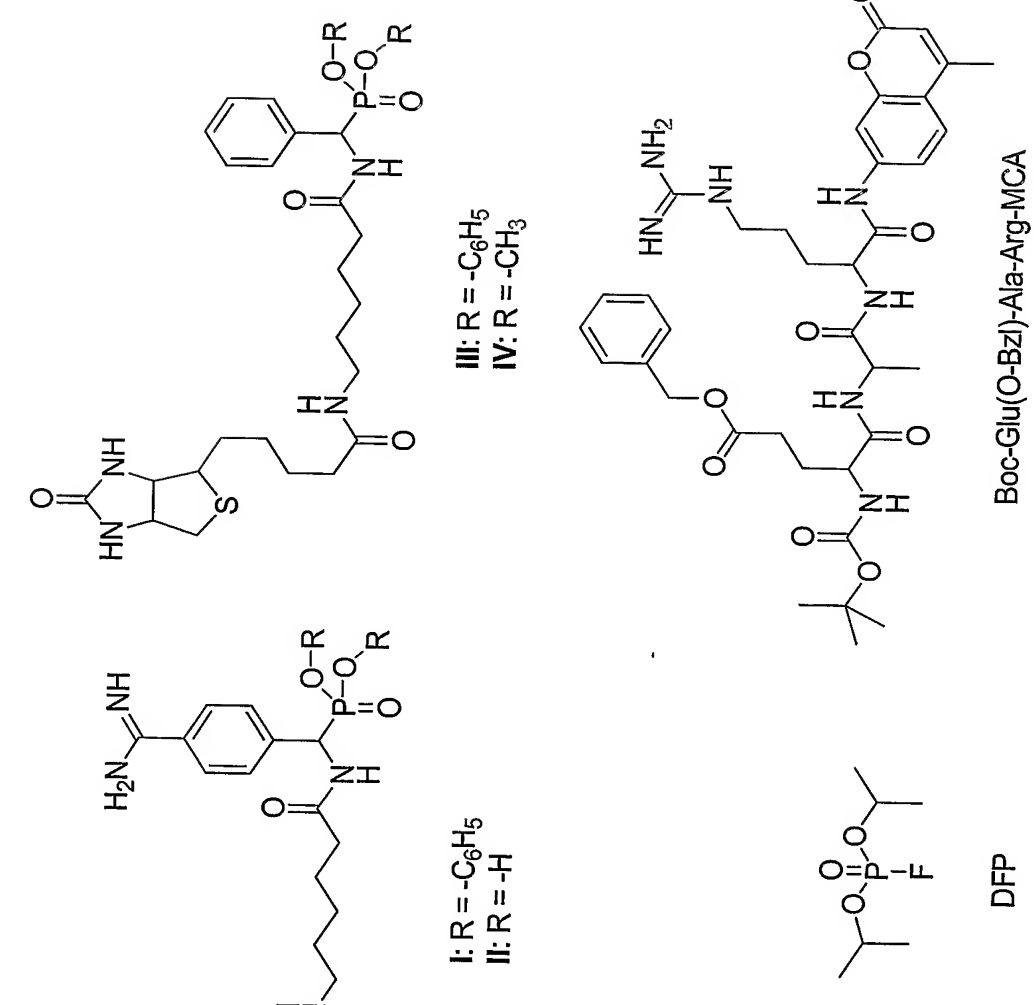


Fig 25

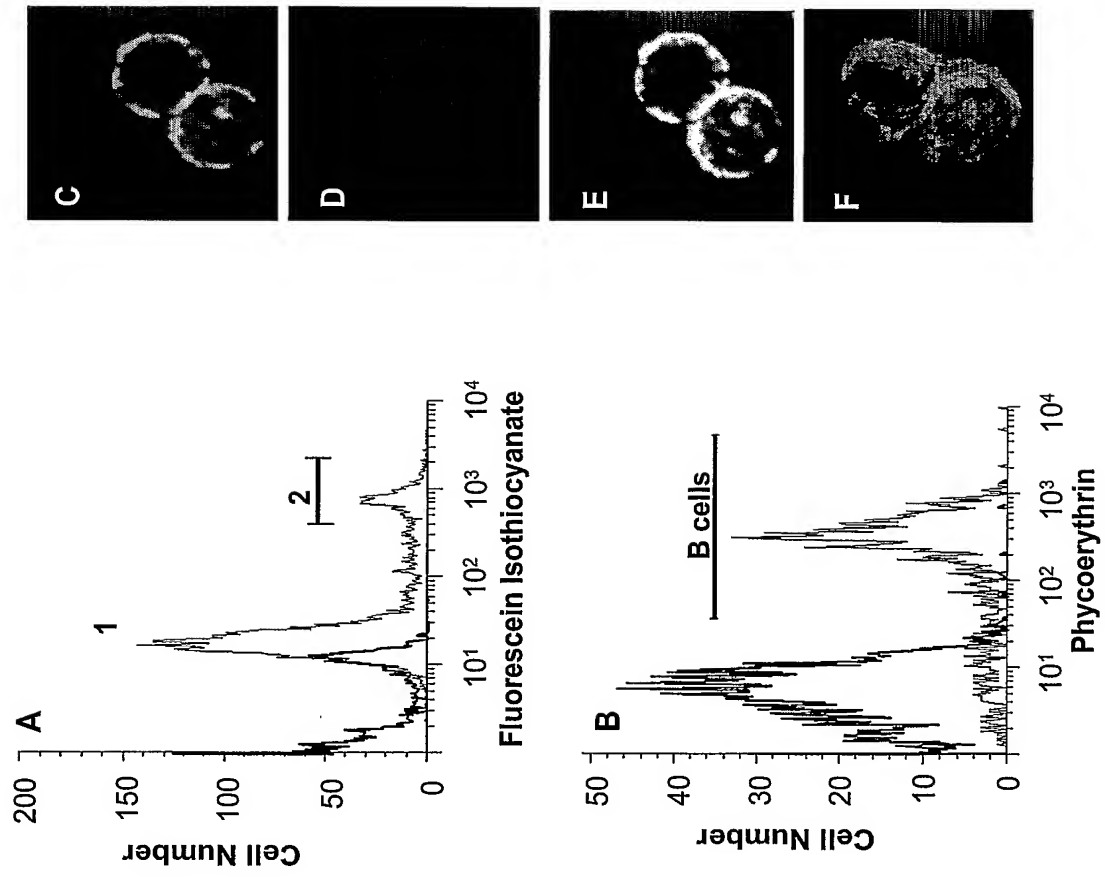

A
1
2
Cell Number
Fluorescein Isothiocyanate
B
B cells
Cell Number
Phycoerythrin
C
D
E
F

Fig 26

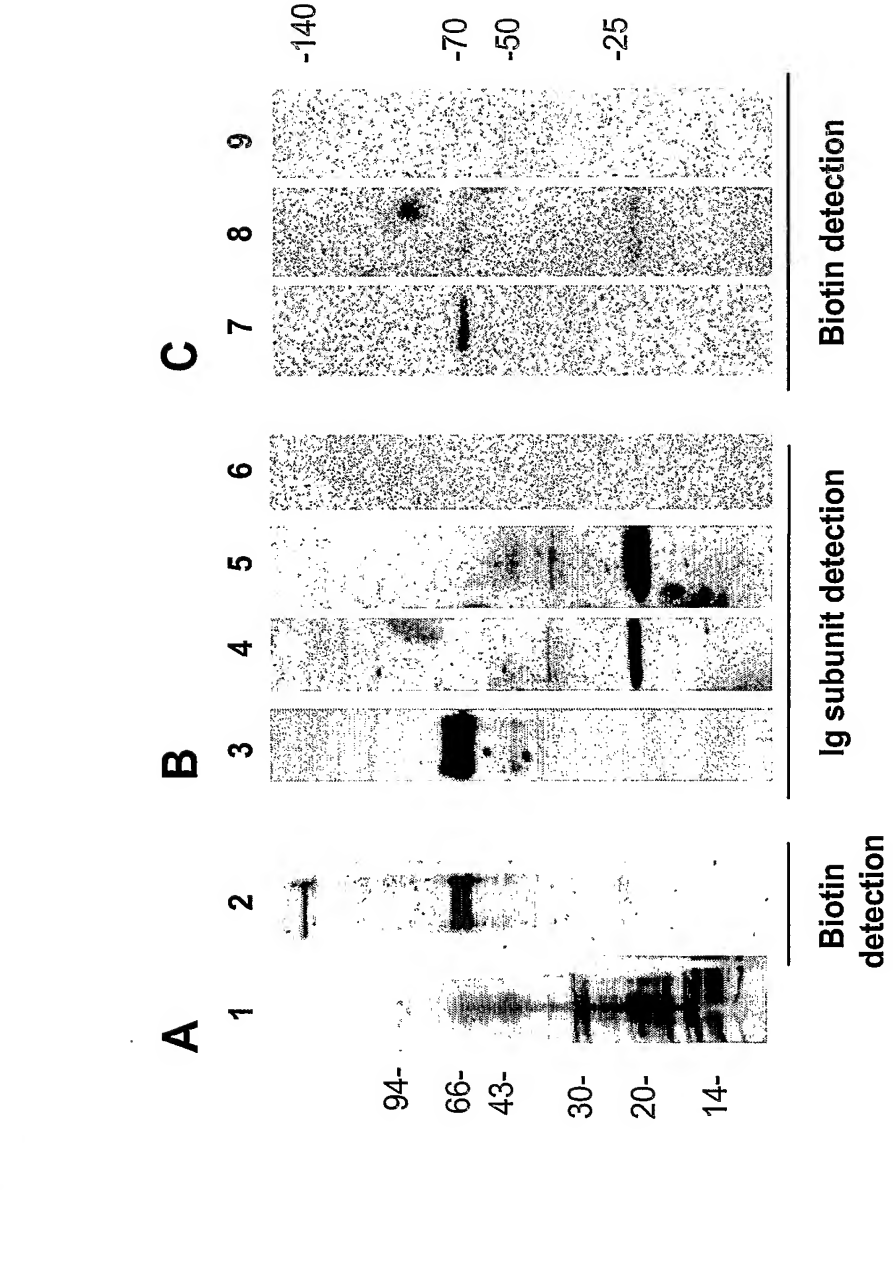
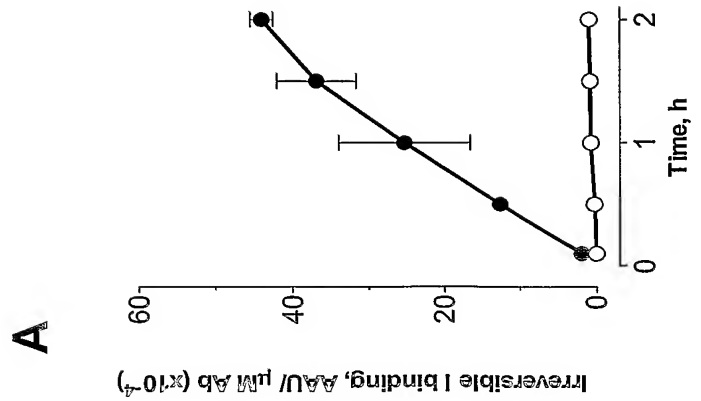
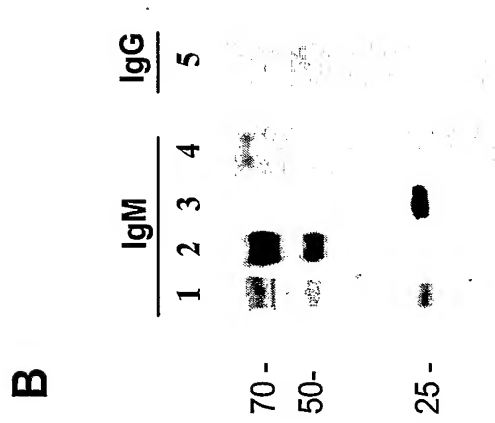

A
1
2
94-
66-
43-
30-
20-
14-
Biotin detection
B
3
4
5
6
Ig subunit detection
C
7
8
9
-140
-70
-50
-25
Biotin detection

Fig 27


A
Irreversible I binding, ΔAU/ μM Ab (x10-4)
60
40
20
0
0
1
2
Time, h


B
IgM
IgG
1 2 3 4 5
70 -
50-
25 -

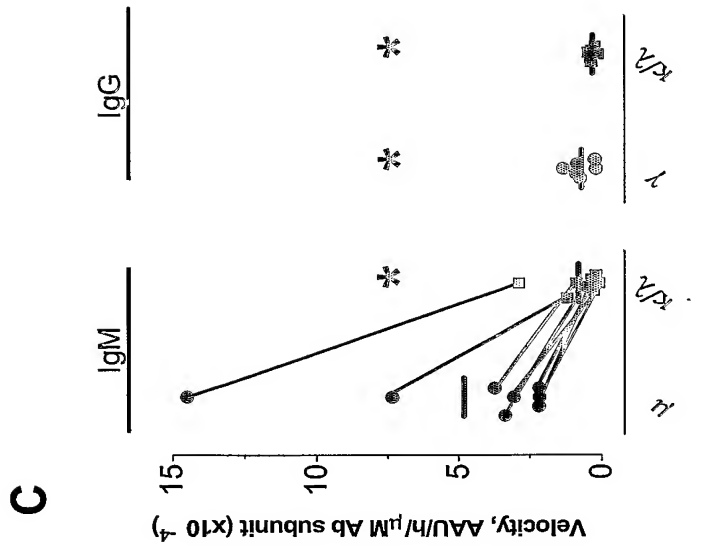

C
IgM
IgG
Velocity, ΔAU/h/μM Ab subunit (x10 -4)
15
10
5
0
κ
λ
Vκ
Vλ

Fig 28

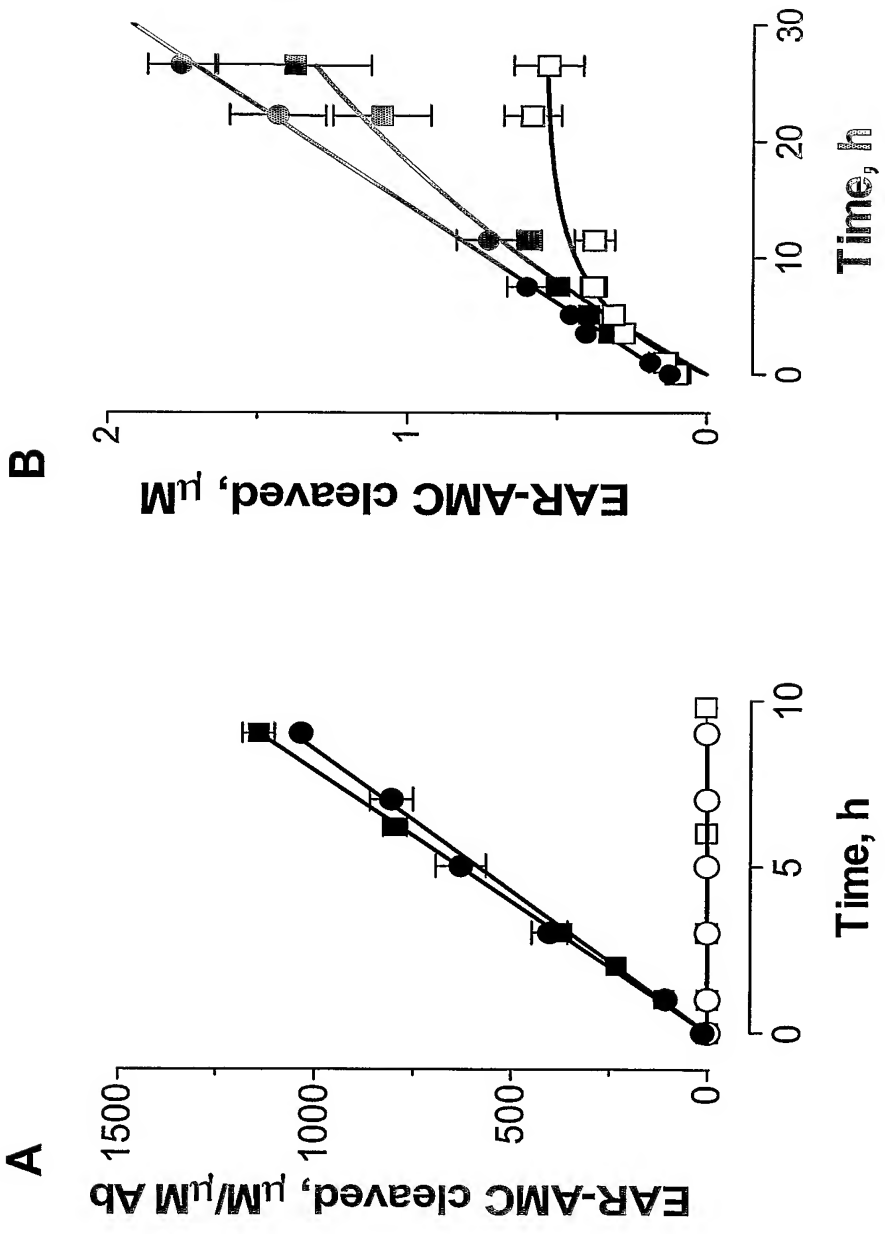
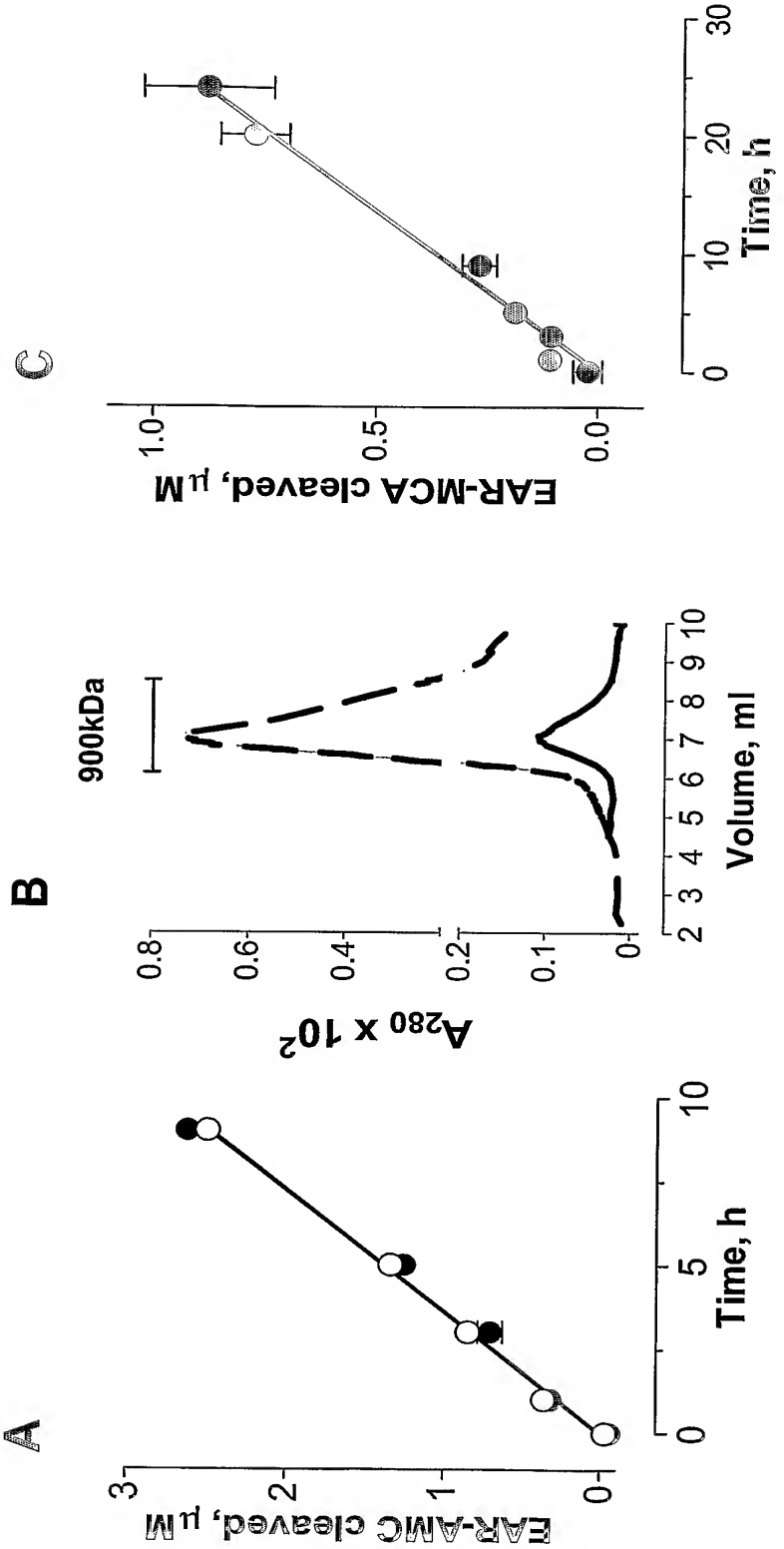

A
EAR-AMC cleaved, μM/μM Ab
1500
1000
500
0
0
5
10
Time, h
B
EAR-AMC cleaved, μM
2
1
0
0
10
20
30
Time, h

Fig 29


A
EAR-AMC cleaved, μM
3
2
1
0
0
5
10
Time, h
B
900kDa
A280 x 10²
0.8
0.6
0.4
0.2
0.1
0
2 3 4 5 6 7 8 9 10
Volume, ml
C
EAR-MCA cleaved, μM
1.0
0.5
0.0
0
10
20
30
Time, h

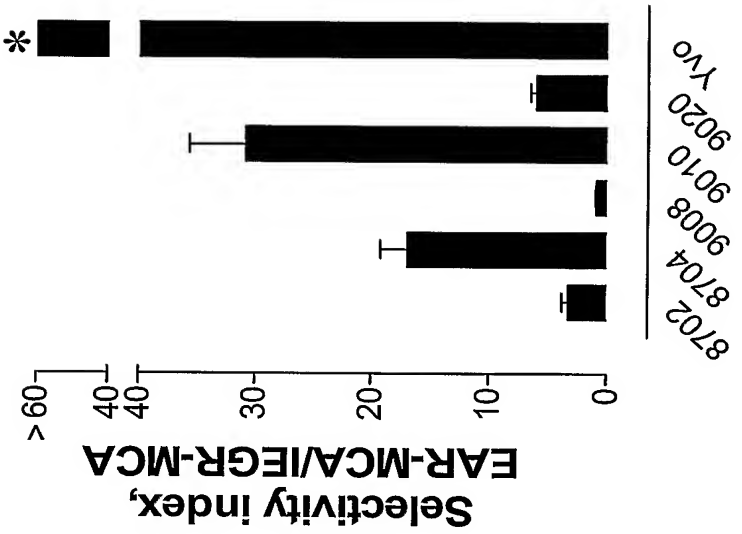

Selectivity index,
EAR-MCA/IEGR-MCA
>60
40
40
30
20
10
0
8702
8704
9008
9010
9020
Vyo
*

Fig 30

Fig 31

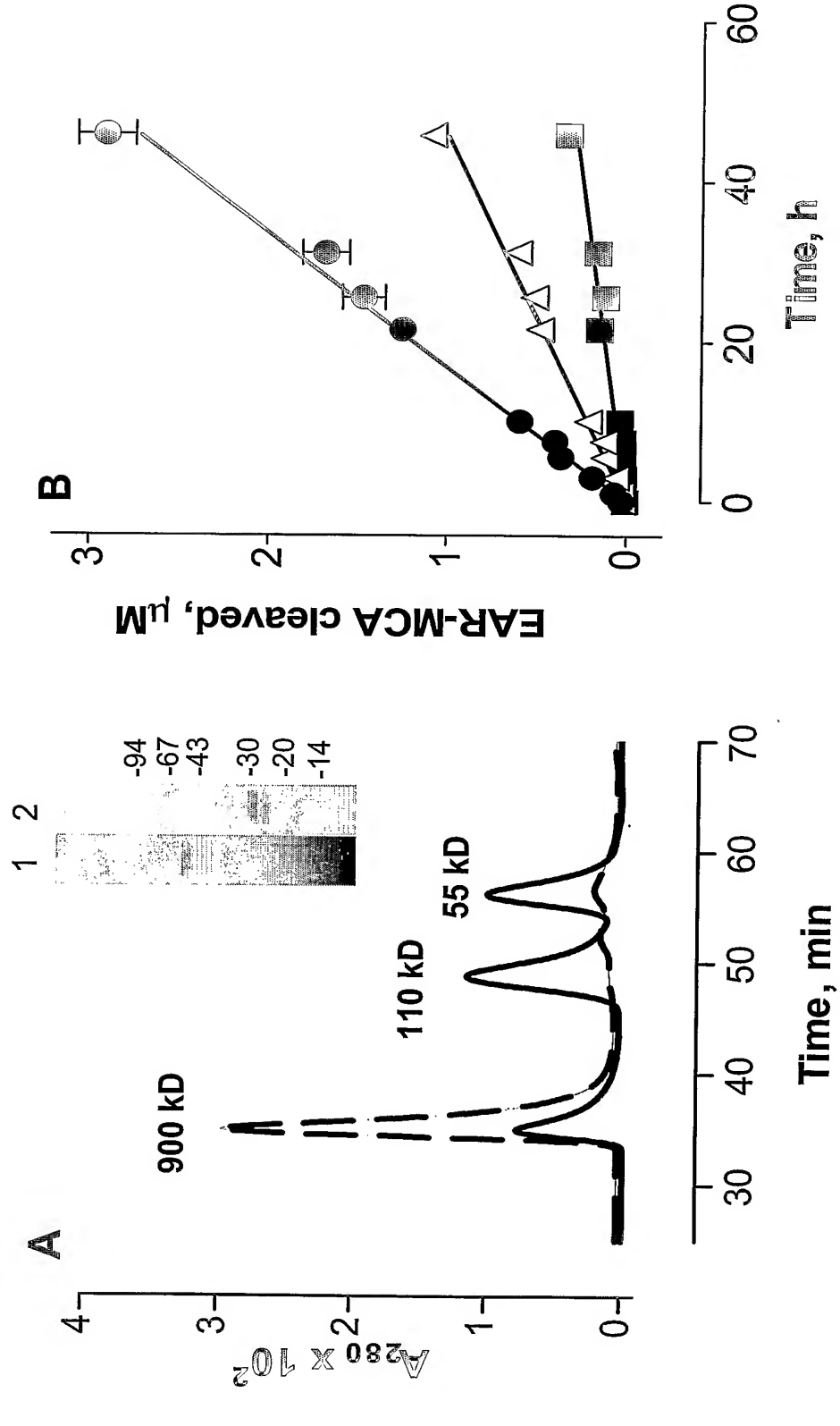
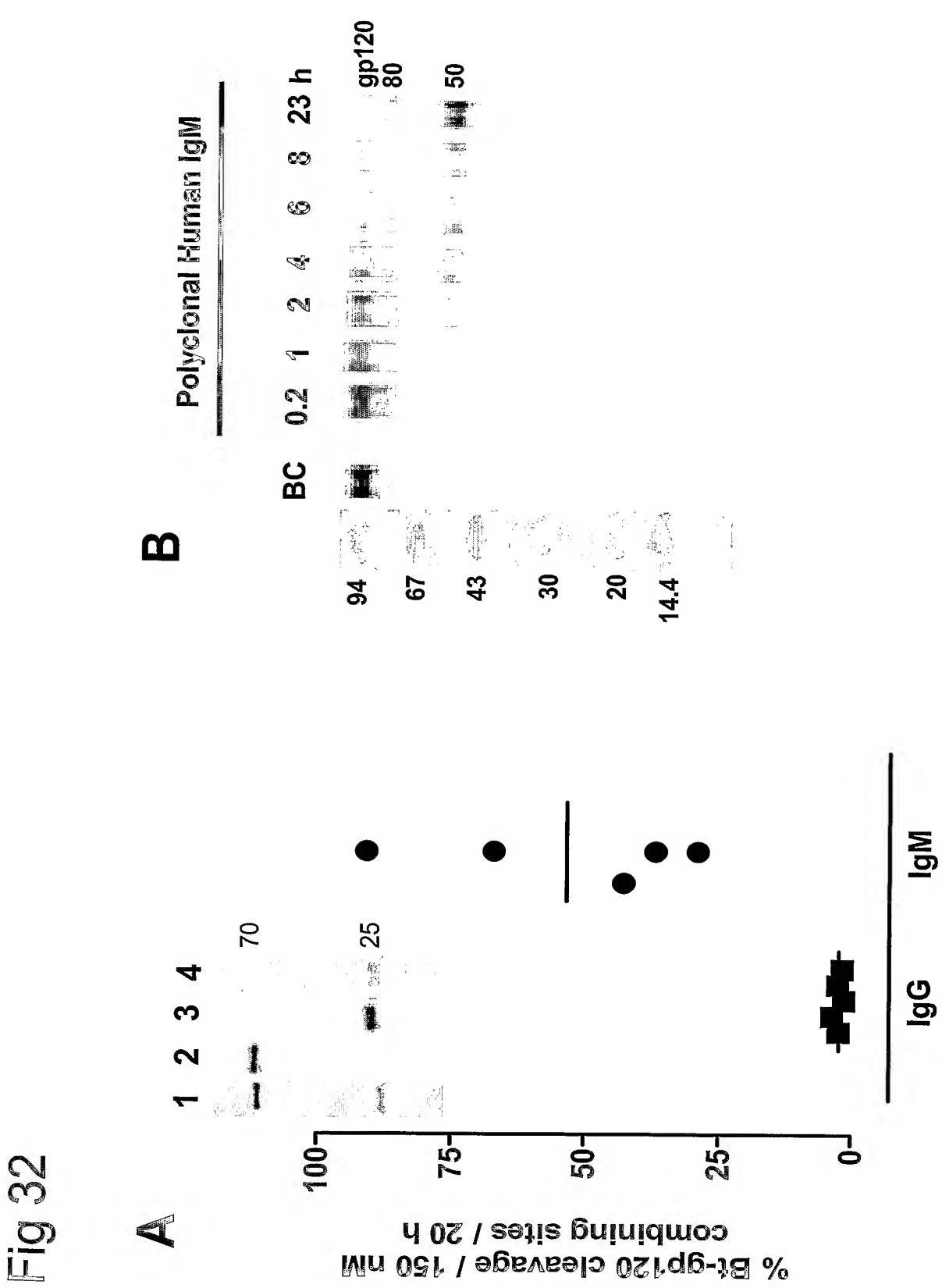

A
B
900 kD
110 kD
55 kD
1 2
-94
-67
-43
-30
-20
-14
$A_{280} \times 10^2$
Time, min
EAR-MCA cleaved, μM
Time, h

Fig 32


A
1 2 3 4
70
25
% Bt-gp120 cleavage / 150 nM
combining sites / 20 h
100
75
50
25
0
IgG
IgM
B
Polyclonal Human IgM
BC 0.2 1 2 4 6 8 23 h
gp120
80
50
94
67
43
30
20
14.4

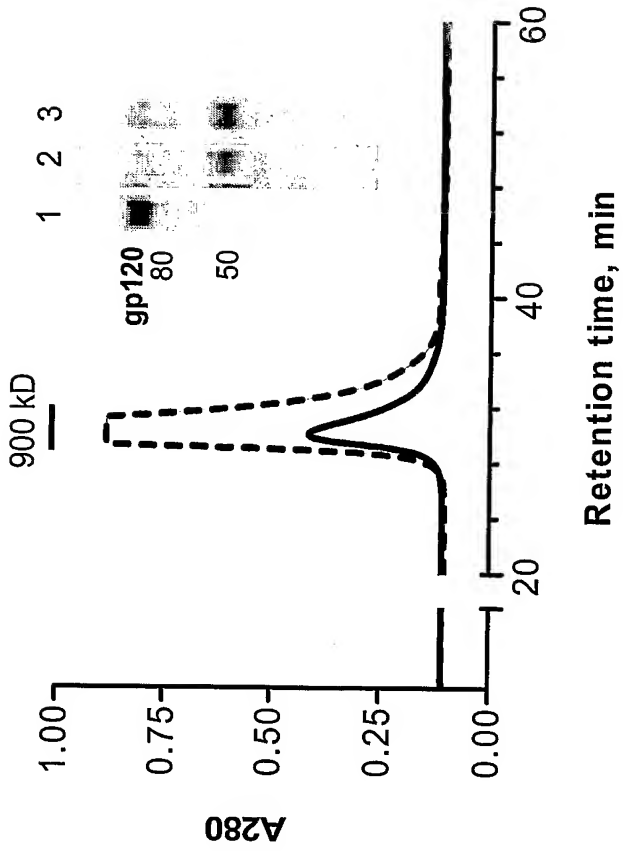

900 kD
1 2 3
gp120
80
50
A280
1.00
0.75
0.50
0.25
0.00
20
40
60
Retention time, min

Fig 33

Fig 34

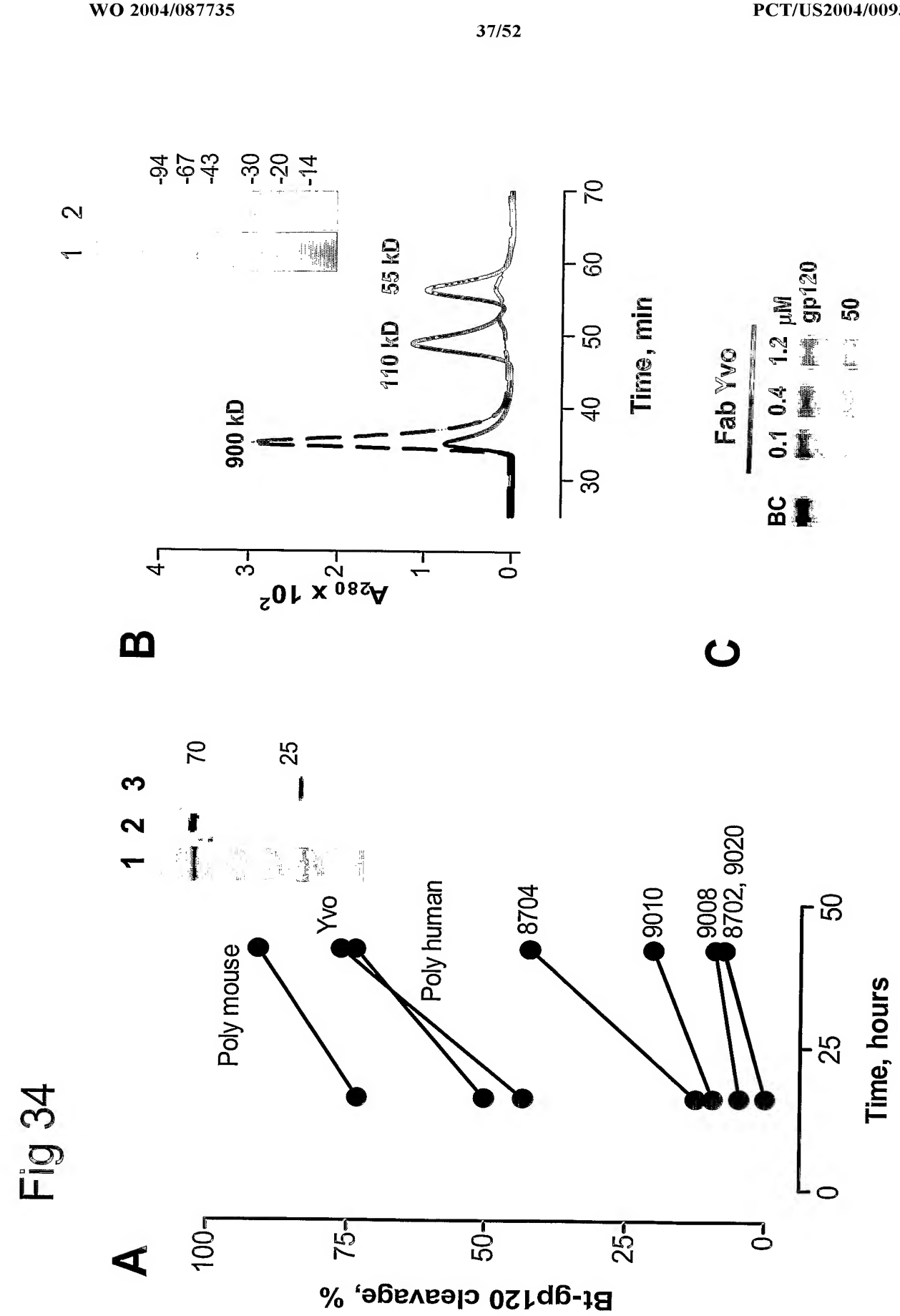
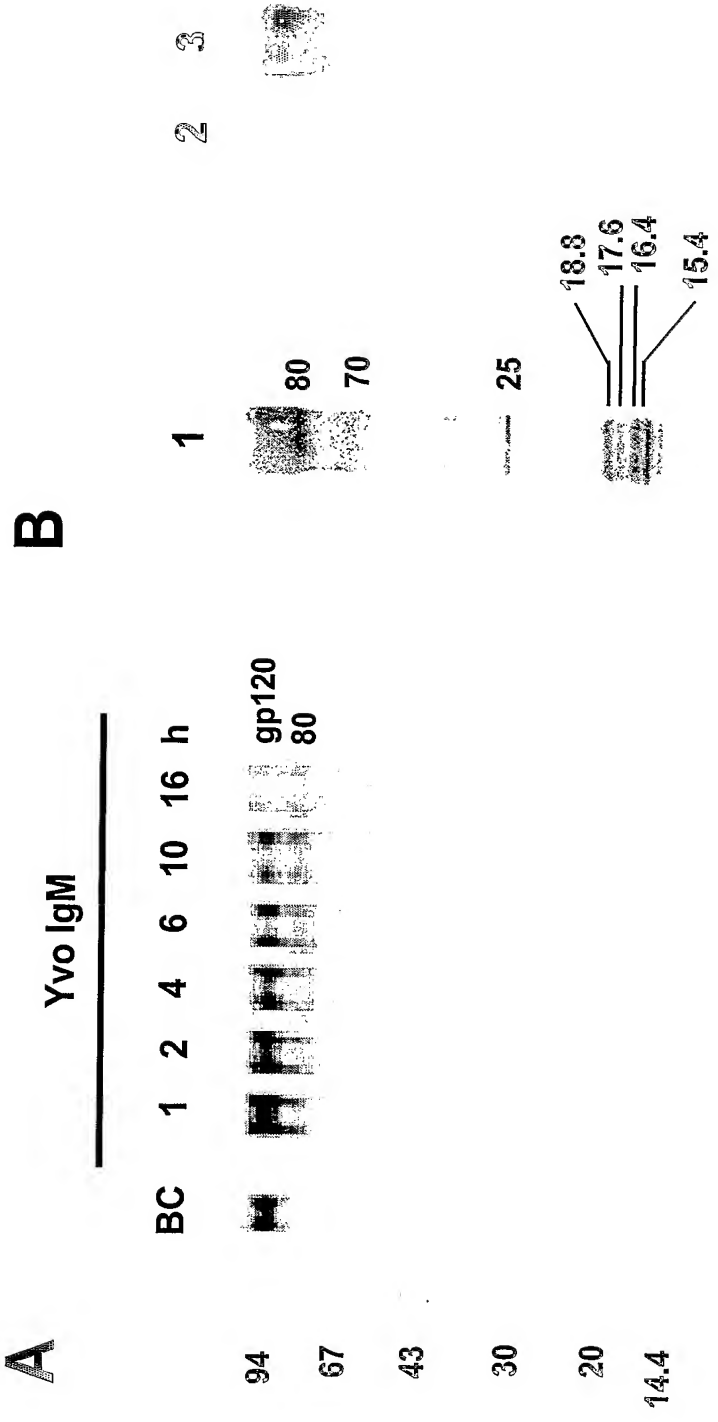

A
Bt-gp120 cleavage, %
100
75
50
25
0
Time, hours
0
25
50
Poly mouse
Yvo
Poly human
8704
9010
9008
8702, 9020
1 2 3
70
25
B
A280 x 10²
4
3
2
1
0
900 kD
110 kD
55 kD
30
40
50
60
70
Time, min
1 2
-94
-67
-43
-30
-20
-14
C
Fab Yvo
BC
0.1 0.4 1.2 μM
gp120
50

Fig 35


A
Yvo IgM
BC
1
2
4
6
10
16
h
gp120
80
94
67
43
30
20
14.4
B
1
2
3
80
70
25
18.8
17.6
16.4
15.4

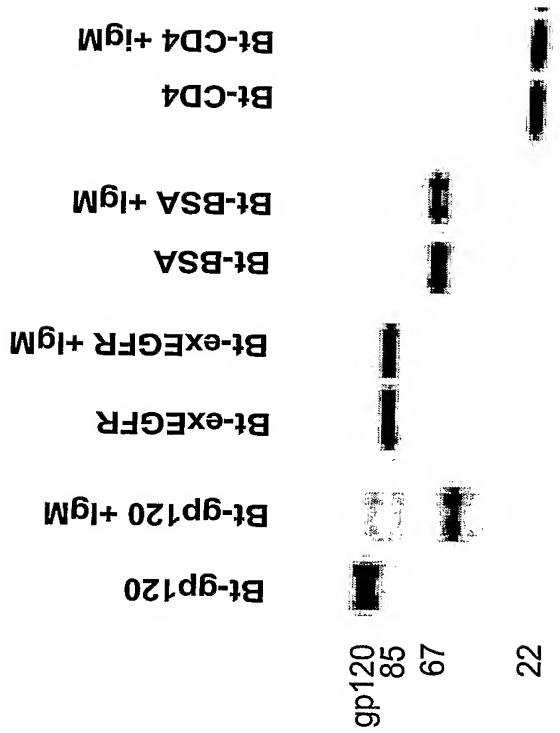

gp120
85
67
22
Bt-gp120
Bt-gp120 +IgM
Bt-exEGFR
Bt-exEGFR +IgM
Bt-BSA
Bt-BSA +IgM
Bt-CD4
Bt-CD4 +IgM

Fig 36

Fig 37

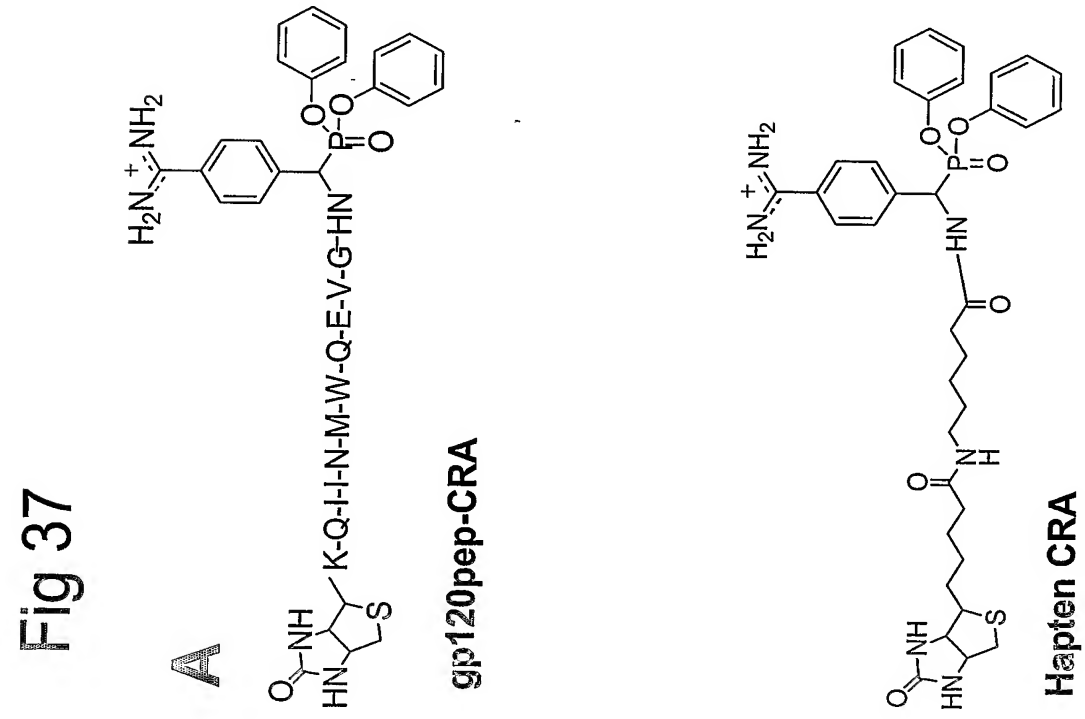

A
gp120pep-CRA
Hapten CRA
B
1 2 3
gp120
80
100
75
50
25
0
Inhibition of Bt-gp120 cleavage, % ± s.d.
0.01 0.1 1 10 100
CRA, μM

Fig 38

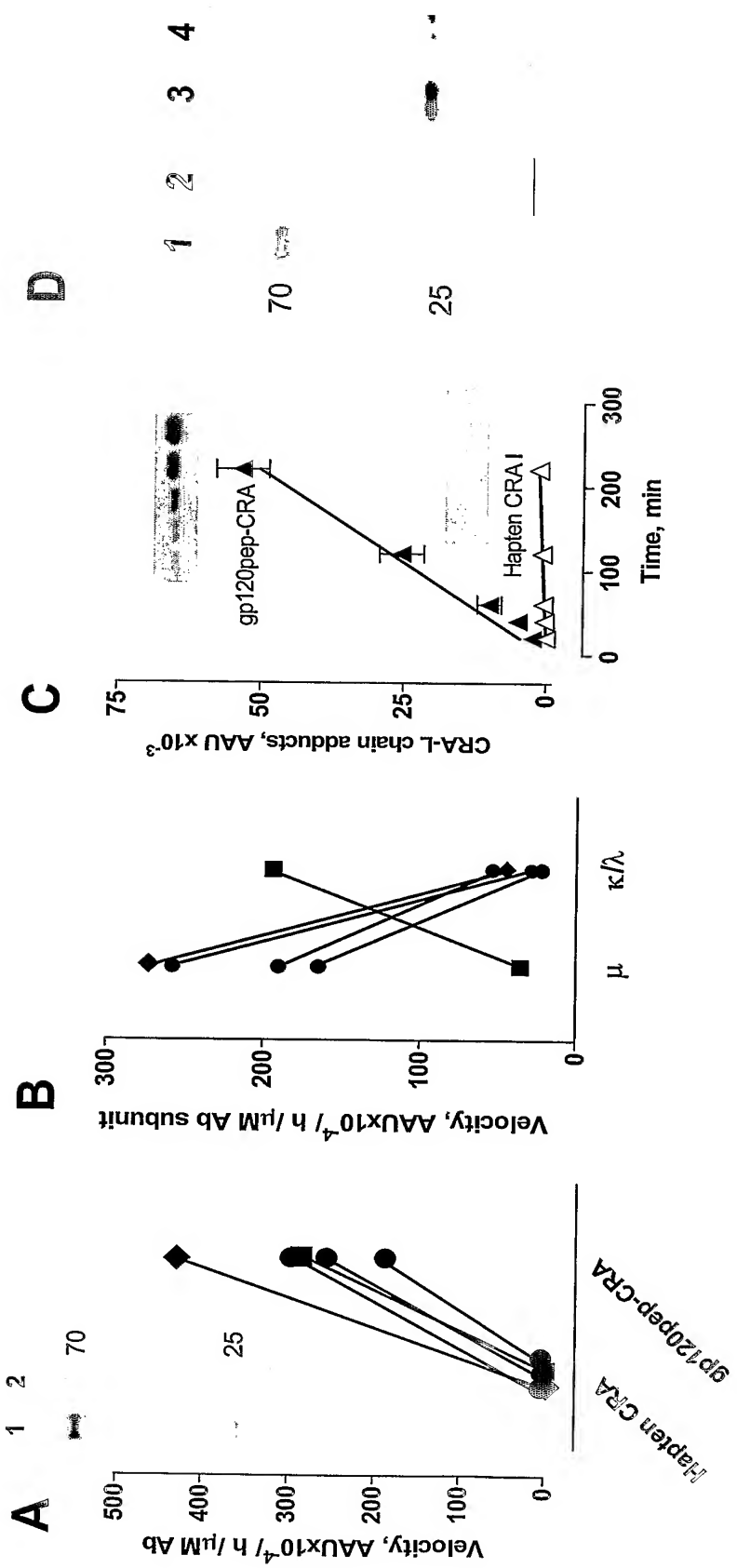
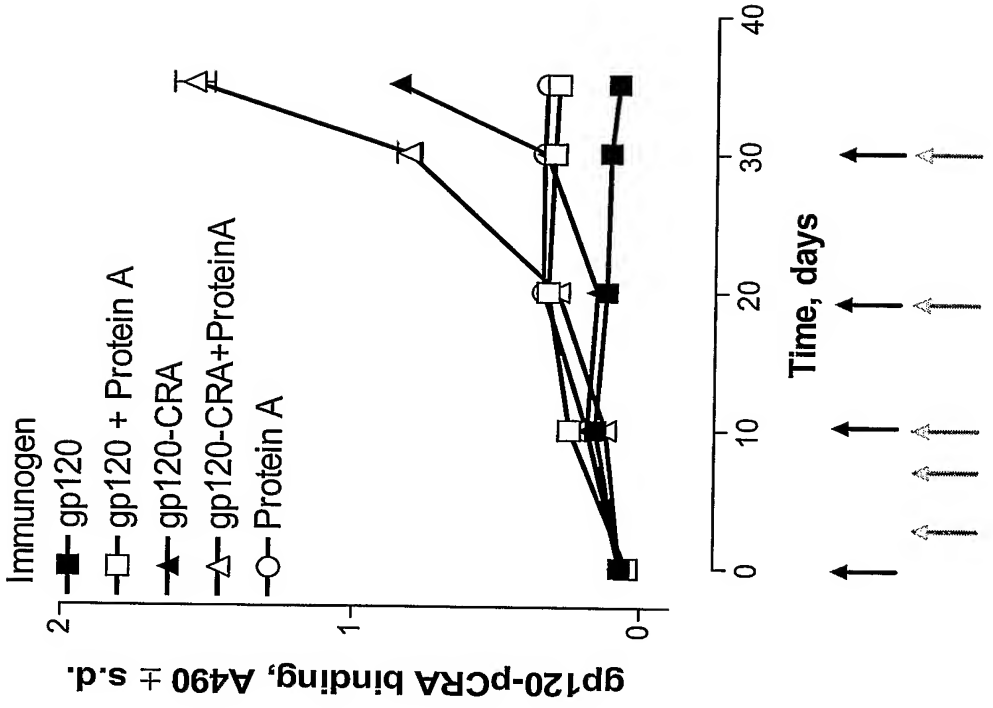

A
1
2
70
25
Velocity, AAUx10⁻⁴/ h /μM Ab
500
400
300
200
100
0
Hapten CRA
gp120pep-CRA
B
Velocity, AAUx10⁻⁴/ h /μM Ab subunit
300
200
100
0
μ
κ/λ
C
CRA-L chain adducts, AAU x10⁻³
75
50
25
0
gp120pep-CRA
Hapten CRA I
0
100
200
300
Time, min
D
1
2
3
4
70
25

Fig 39


gp120-pCRA binding, A490 ± s.d.
2
1
0
Immunogen
gp120
gp120 + Protein A
gp120-CRA
gp120-CRA+ProteinA
Protein A
0
10
20
30
40
Time, days

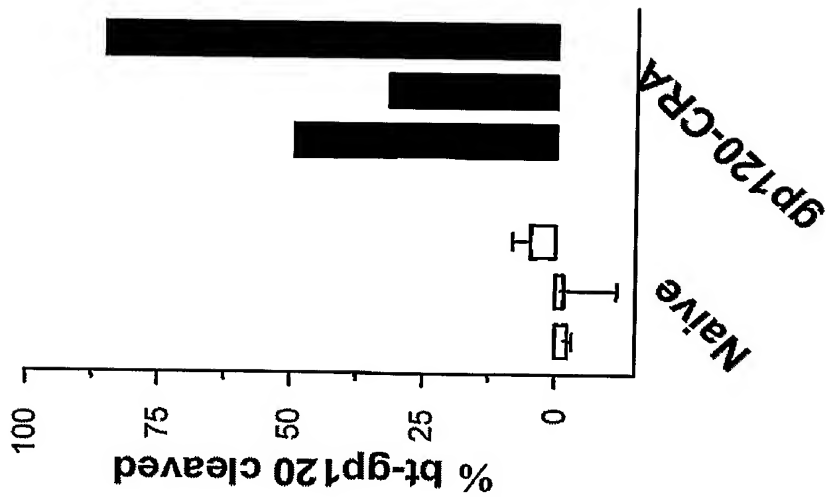


FIG 40

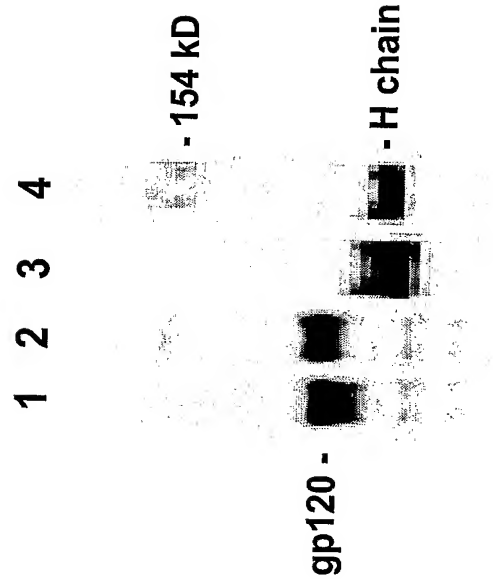


Fig 41

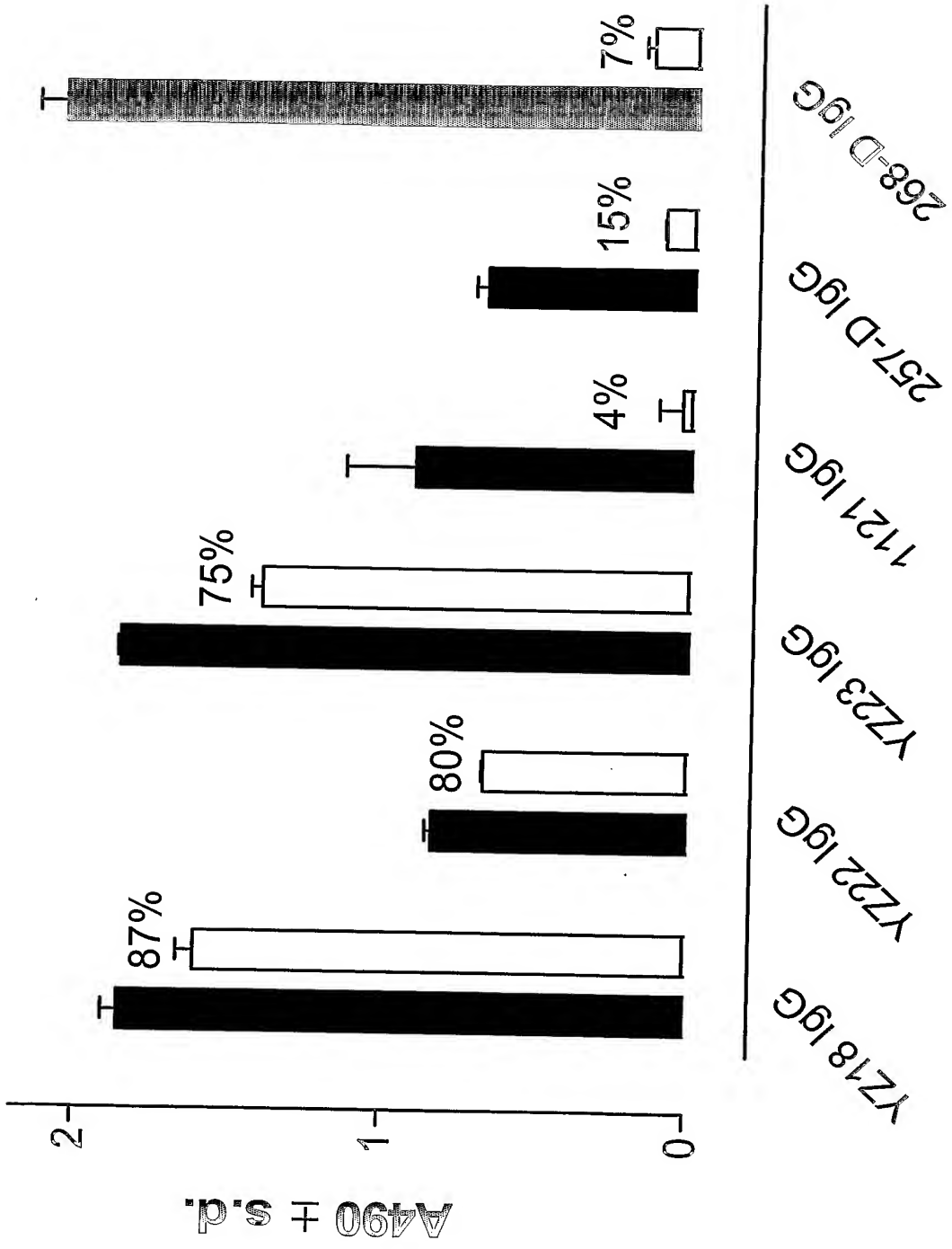

A490 ± s.d.
2
1
0
87%
80%
75%
4%
15%
7%
Y218 IgG
Y222 IgG
Y223 IgG
1121 IgG
257-D IgG
268-D IgG

Fig 42

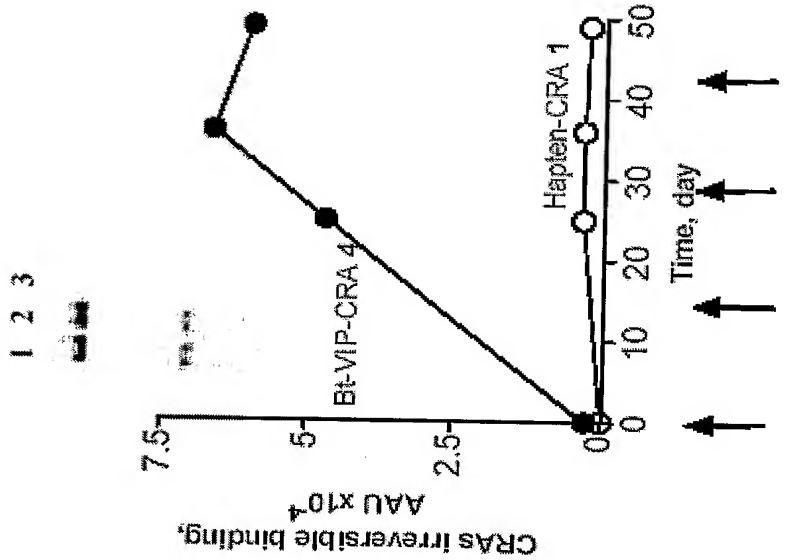

CRAs irreversible binding,
AAU x10⁻⁴
7.5
5
2.5
0
Bt-VIP-CRA 4
Hapten-CRA 1
0
10
20
30
40
50
Time, day
1 2 3

FIG 43

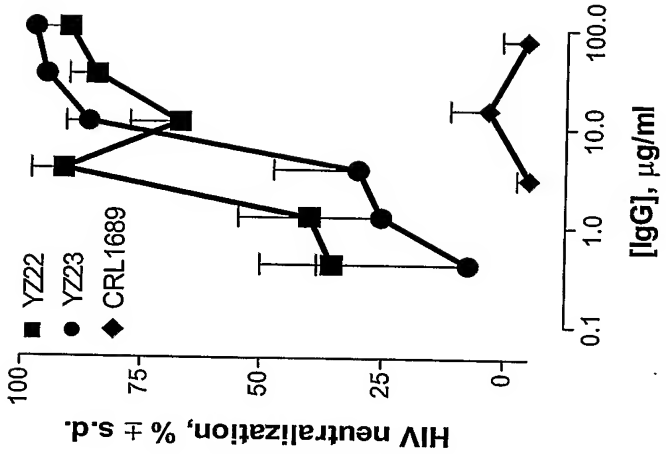
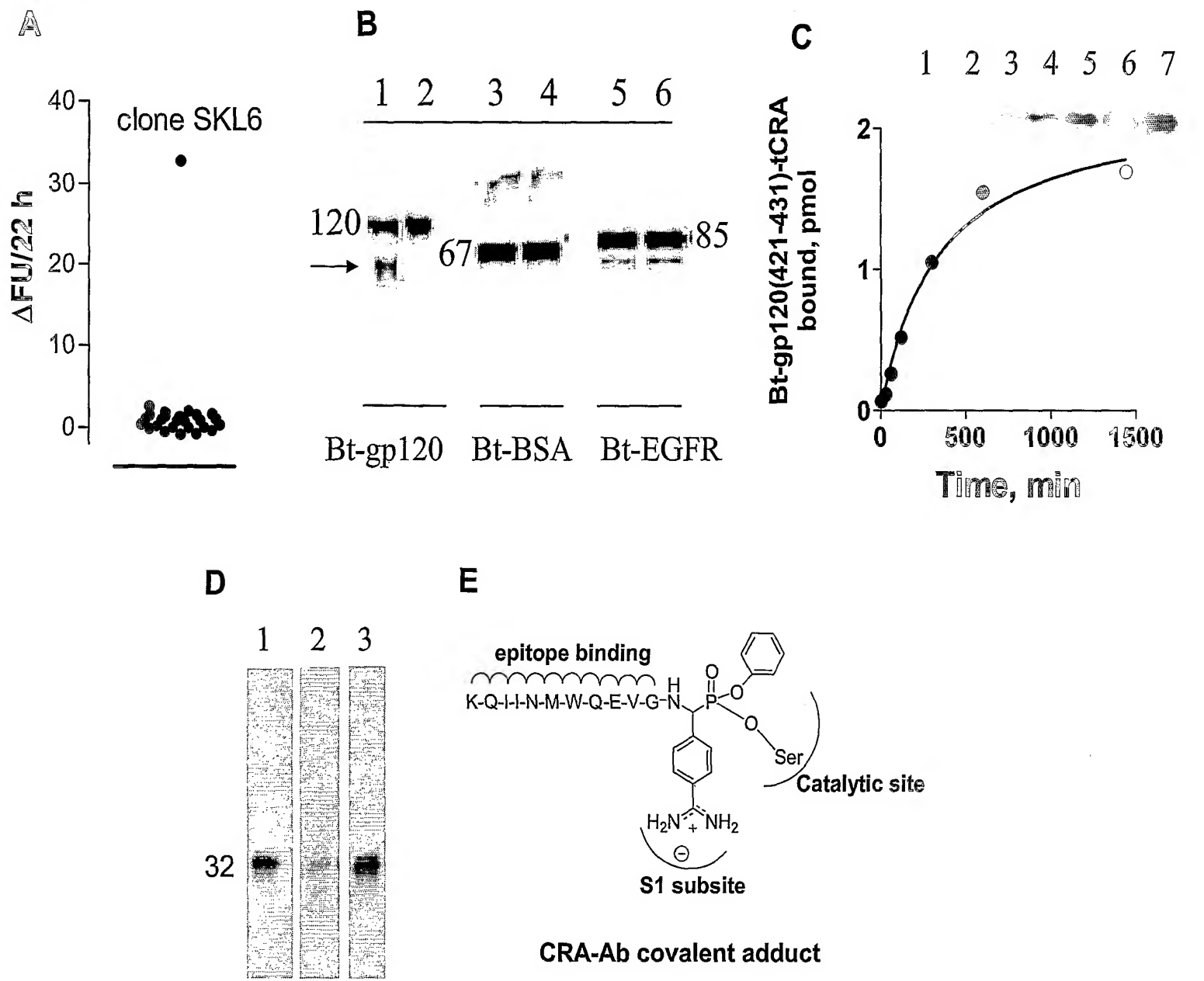

HIV neutralization, % ± s.d.
100
75
50
25
0
YZ22
YZ23
CRL1689
0.1
1.0
10.0
100.0
[IgG], µg/ml

Fig 4

Fig 45


A
clone SKL6
ΔFU/22 h
40
30
20
10
0
B
1 2 3 4 5 6
120
67
85
Bt-gp120
Bt-BSA
Bt-EGFR
C
1 2 3 4 5 6 7
Bt-gp120(421-431)-tCRA bound, pmol
2
1
0
0
500
1000
1500
Time, min
D
1 2 3
32
E
epitope binding
K-Q-I-I-N-M-W-Q-E-V-G-N
H
O
P
O
O
Ser
Catalytic site
H2N
NH2
S1 subsite
CRA-Ab covalent adduct

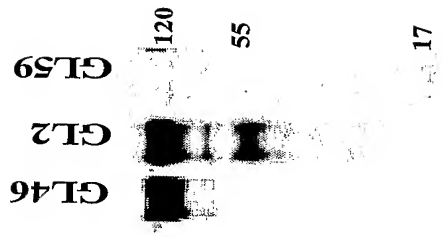


Fig 46

Fig 47

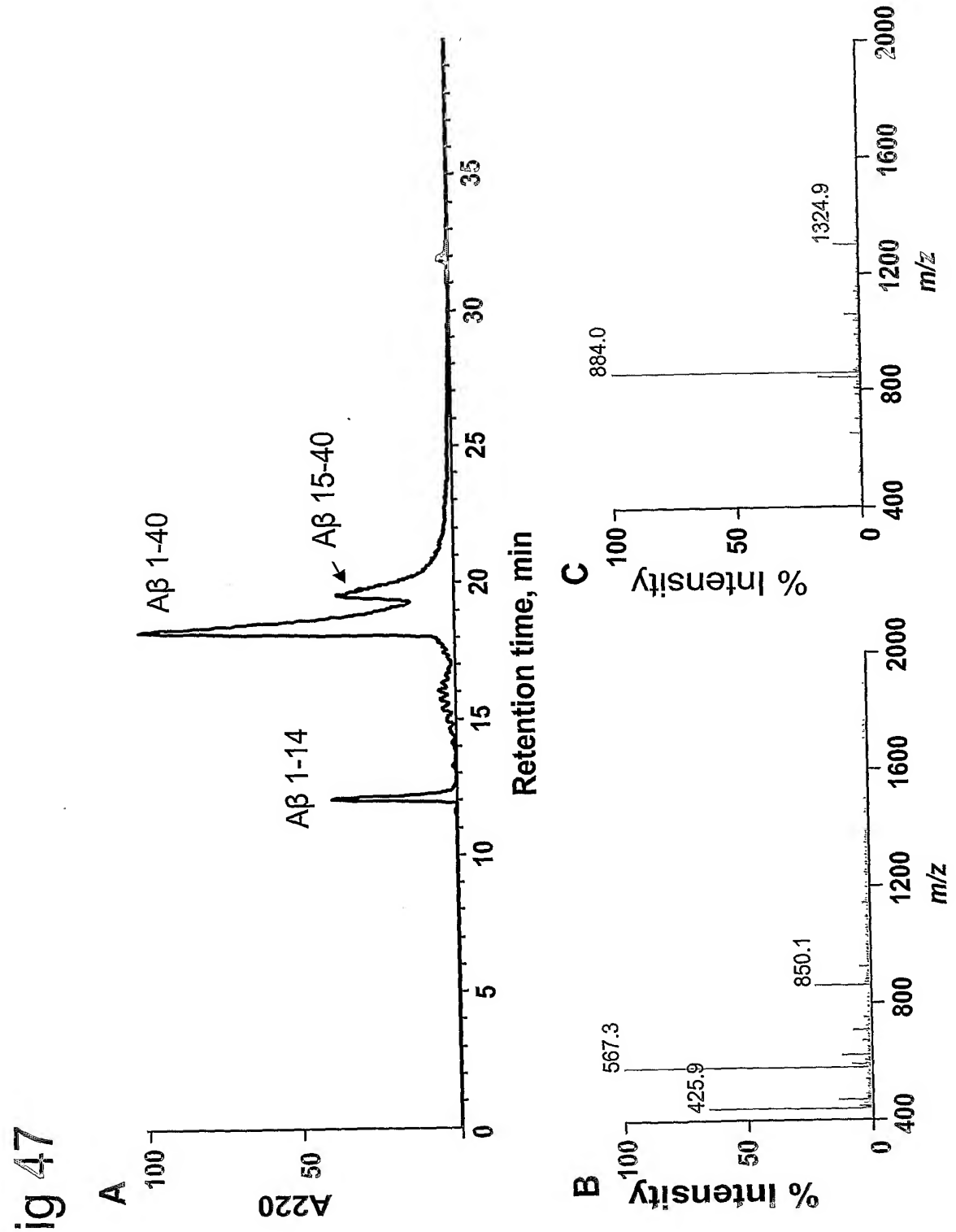
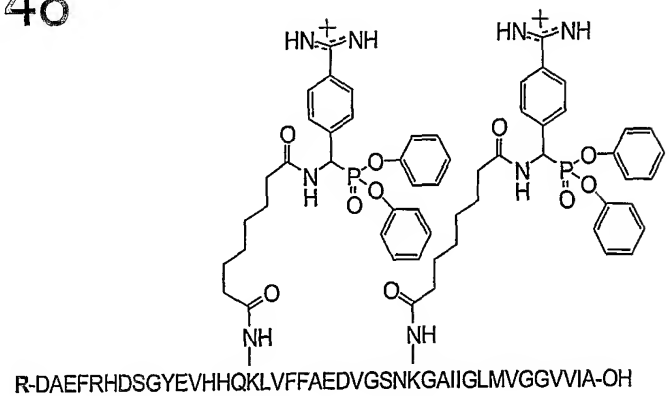
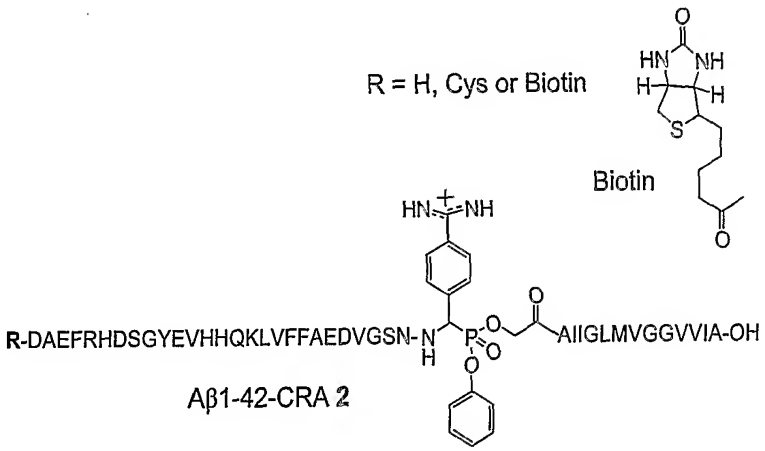

A
A220
100
50
0
Aβ 1-40
Aβ 15-40
Aβ 1-14
0
5
10
15
20
25
30
35
Retention time, min
B
% Intensity
100
50
0
567.3
425.9
850.1
400
800
1200
1600
2000
m/z
C
% Intensity
100
50
0
884.0
1324.9
400
800
1200
1600
2000
m/z

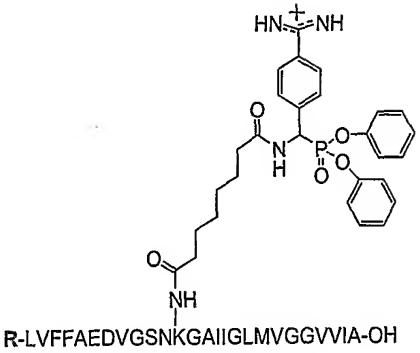
Fig 48



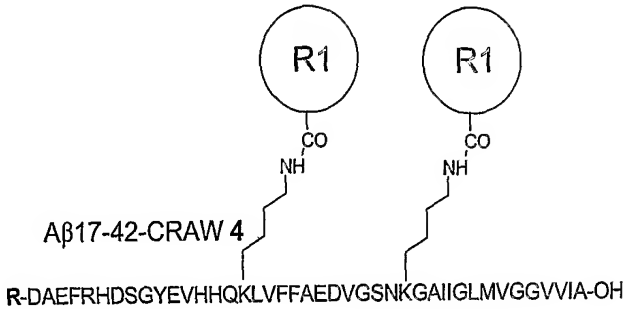
Aβ1-42-CRA **1**



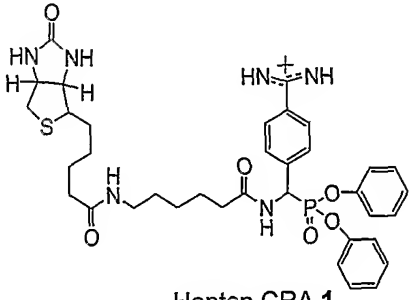
Aβ1-42-CRA **2**



Aβ17-42-CRA **3**



Aβ17-42-CRAW **4**



Hapten CRA **1**

R1 =

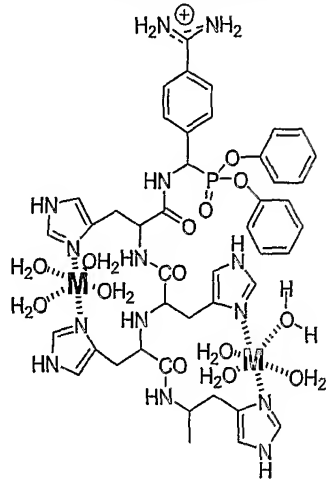
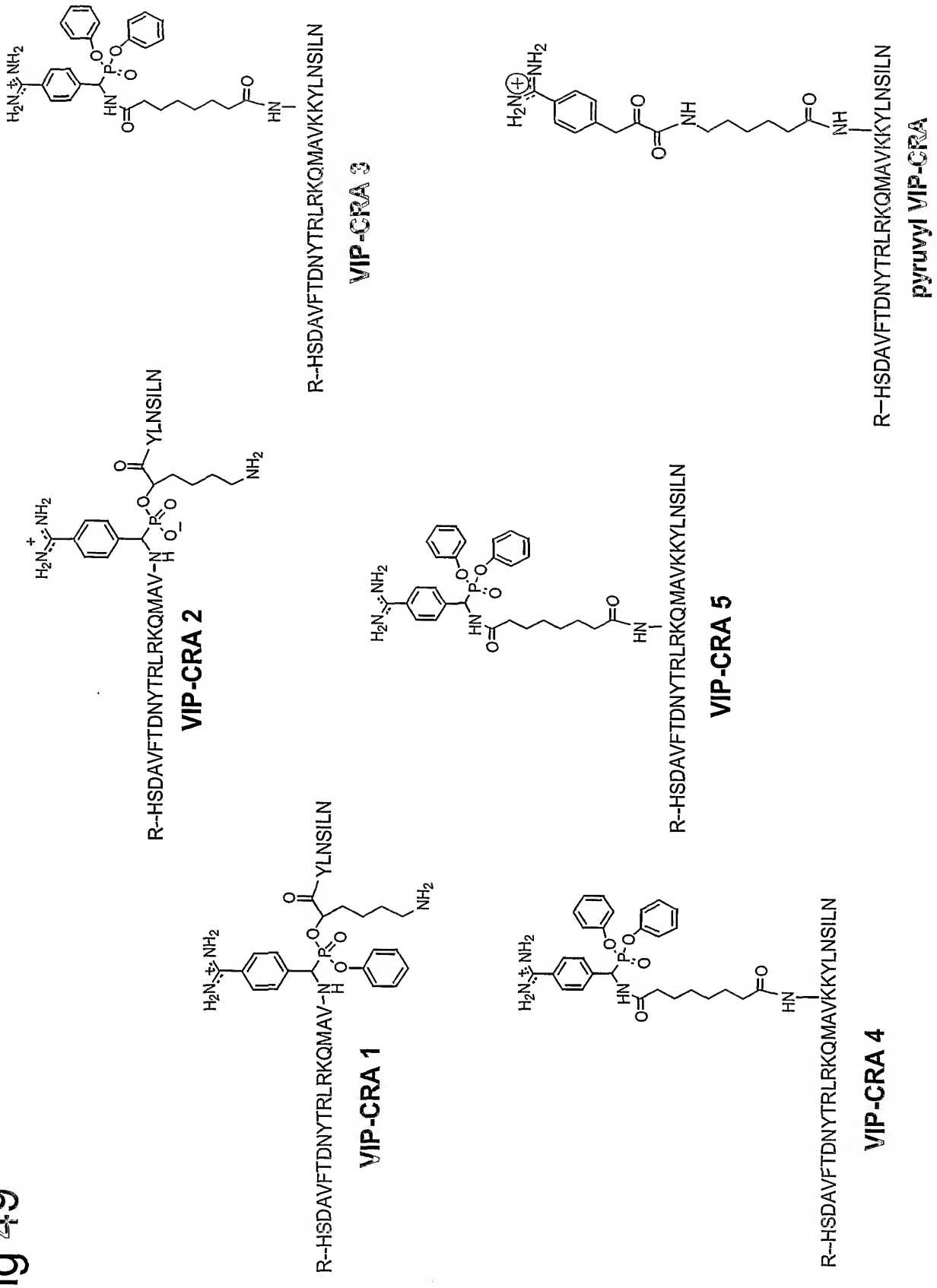


Fig 49


R--HSDAVFTDNYTRLRKQMAV-
YLNSILN
VIP-CRA 1
R--HSDAVFTDNYTRLRKQMAVKKYLNSILN
VIP-CRA 2
R--HSDAVFTDNYTRLRKQMAVKKYLNSILN
VIP-CRA 3
R--HSDAVFTDNYTRLRKQMAVKKYLNSILN
VIP-CRA 4
R--HSDAVFTDNYTRLRKQMAVKKYLNSILN
VIP-CRA 5
R--HSDAVFTDNYTRLRKQMAVKKYLNSILN
pyruvyl VIP-CRA